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Development of Biological Indices to Pollution Levels in Streams Affected by Acid Mine Drainage and Oil Field Brine Wastes

February, 1969

State of Ohio
Water Resources Center
Ohio State University

DEVELOPMENT OF BIOLOGICAL INDICES TO POLLUTION LEVELS IN STREAMS AFFECTED BY ACID MINE DRAINAGE AND OIL FIELD BRINE WASTES

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Natural Resources Institute and the
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Research Project Completion Report
Project No. A - 003 - OHIO

Submitted to: Office of Water Resources Research
The United States Department of the Interior

February, 1969

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INTRODUCTION AND PROJECT OBJECTIVES

Enactment of the Water Quality Act of 1965 by the United States Congress is expected to lead to wide adoption of water quality standards for natural waters of the country which assure, among other goals, waters capable of supporting desirable levels of aquatic life. Although criteria have been developed for establishing water quality standards for public health and certain other domestic and industrial uses, they are not well developed for many aquatic organisms. Further, there are few reliable and readily applicable indices by which the quality of water for aquatic life purposes may be continuously assessed in natural waters. The major purpose of this study is to contribute to the development of biological criteria which may find practical applications in the adoption of water quality standards for the assurance of desirable levels of aquatic life in naturally flowing streams. This study has added significantly to the body of information extant for such application. It has contributed significantly also to the training of biologists competent in this area.

Sophisticated stream monitoring devices have been developed in recent years which permit continuous measurement and recording of a limited number of critical parameters at specific points. The number of such devices in use or planned for installation is increasing. There appears, however, to be little prospect in the forseeable future that they will be available in sufficient number or range of capability for measuring all of the factors involved in determining water quality for aquatic life.

Quantitative and qualitative studies of aquatic organisms provide a measurement of water quality over an extended period of time because of the variations in tolerance to specific environmental conditions exhibited by certain species. Certain bottom feeding organisms, such as Mayflies (Hexagenia spp. Ephoron album (Say) and Ephemera simulan Walker), for example, appear abundantly only under conditions of continuous high oxygen availability, whereas certain midges (Chironomidae) become dominant under near anaerobic conditions. Certain vertebrate organisms also reflect changes in water quality. Ecological study of streams and impoundments provide a useful tool for detecting water quality characteristics inimical to certain uses and a guide to the degree of treatment of watershed modification necessary to achieve optimum water utilization. The method is readily adaptable to waters of varying characteristics; it is mobile, and a direct measure of water quality related to aquatic production is provided.

This study was undertaken to measure the relationship between populations of naturally occurring aquatic organisms and variations in water quality as measurable by known physical and chemical techniques in stream systems known to be adversely affected by specific pollutants. In so far as possible all aspects of the ecosystem which impinge on water quality were studied.

ACKNOWLEDGEMENTS

One of the first studies undertaken at The Ohio State University through the allotment program authorized by the Water Resources Research Act was an investigation of biological indices to pollution from acid mine drainage and brine wastes from oil field operations. The results of these studies are reported here. This project, known as Ohio State University Water Resources Center Project 103, was initiated in April, 1965, and concluded June 30, 1967.

The investigators wish to acknowledge the importance of and express appreciation for the financial and technical support for the project (A-003-OHIO) made possible through the allotment program administered by the Office of Water Resources Research in the U. S. Department of the Interior through The Ohio State University Water Resources Center. We wish especially to express appreciation to Professor George P. Hanna, Jr., Director of the Ohio State University Water Resources Center; to Jane Mahan, of his office staff; and Kenesaw Shumate, of his laboratory staff, for their prompt and untiring efforts to aid the project in every feasible way. We were able, when necessary, to utilize office and laboratory space and equipment in the Center, to use the Center as a base of operations, as an equipment staging area, and a center for project coordination.

A total of four faculty members and nine advanced students participated in the project. Two of the faculty members were from Ashland College. We wish to express special appreciation to Miss Dorothy Adalis of Ashland College, Mr. James T. Addis, graduate student at The Ohio State University and fisheries specialist for the Ohio Division of Wildlife, and Dr. C. E. Taft of the Botany Department of The Ohio State University, who had important roles in supervising student work on the project and assisting with the taxonomy of organisms. The students who participated are deserving of special thanks for their industry and their willingness to adjust work schedules to meet project requirements. Mssrs. Dale Anderson, Richard Christensen, Gary W. Hackett, James R. Heikel, Charles R. Schwartz, John R. Steinback, Stanley Wirt, and Arnold Woodrich are deserving of such recognition. Finally, the undersigned wishes to recognize the special role in this project taken by Dr. John H. Olive of Ashland College. Dr. Olive supervised the field and laboratory work; he compiled the data into usable form, and has largely been responsible for preparation of the report. The undersigned has served mostly as an innovator and expediter of the project.

Charles A. Dambach
Principal Investigator

RATIONALE

Numerous studies during the past 60 years have shown the value of using biological criteria for assessing the quality of natural waters (Kolkwitz and Marrson 1908, 1909; Carpenter 1924; Richardson 1921, 1928; Farrell 1931; Jones 1940a, 1940b; Lackey 1939; Butcher 1947, 1955; Gaufin and Tarzwell 1952, 1956; Paine and Gaufin 1956; Patrick 1949, 1957; Hynes 1960, 1962; Wilm and Dorris 1966). The use of biological criteria compliment the more conventional chemical and physical means of water quality analysis and in many cases offer distinct advantages over abiotic methods.

For example, rivers vary considerably in their susceptability to pollution. A load of organic matter that would severely damage the biocommunities in a trout stream may cuase little or no effect on the organisms of a sluggish base-level river (Hynes 1962). Also chemical surveys indicate stream conditions only at the time of sampling and highly concentrated "slugs" of toxic wastes may be completely undetected. In addition, chemicals that affect water quality are so numerous, vary so much in concentration and produce such a wide range of effects because of variations in temperature, pH, turbidity, velocity, and other physical factors that a satisfactory evaluation based solely on physico-chemical methods appears unrealistic.

The biological community on the other hand, monitors the environment continually, reflecting in its genetic composition, metabolism, and species structure the interaction of all environmental parameters. Two approaches are commonly employed in tthe study of biocommunities. One emphasizes biomass and production, dealing with the communities in terms of matter and energy, and the other emphasizes structure. In the latter approach, communities of plants and animals are regarded as complexes of individuals belonging to different species with definite ecological requirements. The structure of biocommunities may be described by the number of species per unit area, species frequency, relative abundance of species, and various biotic and species diversity indexes (Fisher, et al. 1943; Preston 1948; Patrick 1949, 1954; Beck 1955; Margalef 1956; Hairston 1959; Beak 1964; Patten 1962; Wilm and Dorris 1966; MacArthur 1965).

The benthic macroinvertebrate and diatom communities are especially useful for biolgical analyses because the structure of these communities are sensitive to environmental change, yet stable and immobile enough for accurate measurements.

This study deals with the biocommunity structure of polluted streams. The benthic macroinvertebrate and algal communities of (1) Raccoon Creek in Southeastern Ohio and of (2) two headwater tributaries of the Scioto River in central Ohio are described in terms of the relative abundance of certain organisms and in the species diversity index, $\bar{H} = - \Sigma(n_i/N)\log_2(n_i/N)$ (Wilm and Dorris 1966). An inventory of organisms collected is also included. The biological information then is related to the physico-chemical characteristics of each stream.

STUDY AREAS

Olentangy - Whetstone

The upper Olentangy River and Whetstone Creek are permanent headwater streams of the Scioto River system located in central Ohio (Fig. 1). Together, they drain approximately 877 square km of land of which 85 per cent is cropland and pasture, ten per cent woodland, and and four to five per cent urban. The Olentangy arises near Galion at an elevation of 362 m, flows SSW for approximately 78 km, and empties into Delaware Reservoir at an elevation of 272 m. From the Delaware Reservoir, it continues for approximately 80 km, joining the main channel of the Scioto River in Columbus. The latter portion of the stream was not studied. Whetstone Creek originates in farmland 15 km north of Mt. Gilead at an elevation of 395 feet, flows SSW for approximately 53 km and also empties into Delaware Reservoir.

Soils in both watersheds are of glacial origin. Upland areas are predominantly associations of Blount, Pewamo, and Morley soils, characterized by fine texture, and inadequate drainage, but capable of moderate to high productivity. Headwater sections contain moderate areas of Bennington, Marengo, and Cardington soils which are similar to the Blount, Pewamo, Morley associations, but usually are capable of greater productivity. Flood plains of both streams contain Genesee, Eel, Ross and Sloan soil complexes characterized by good drainage (except for Sloan soil) and capable of very high productivity (Ohio Division of Water, 1963).

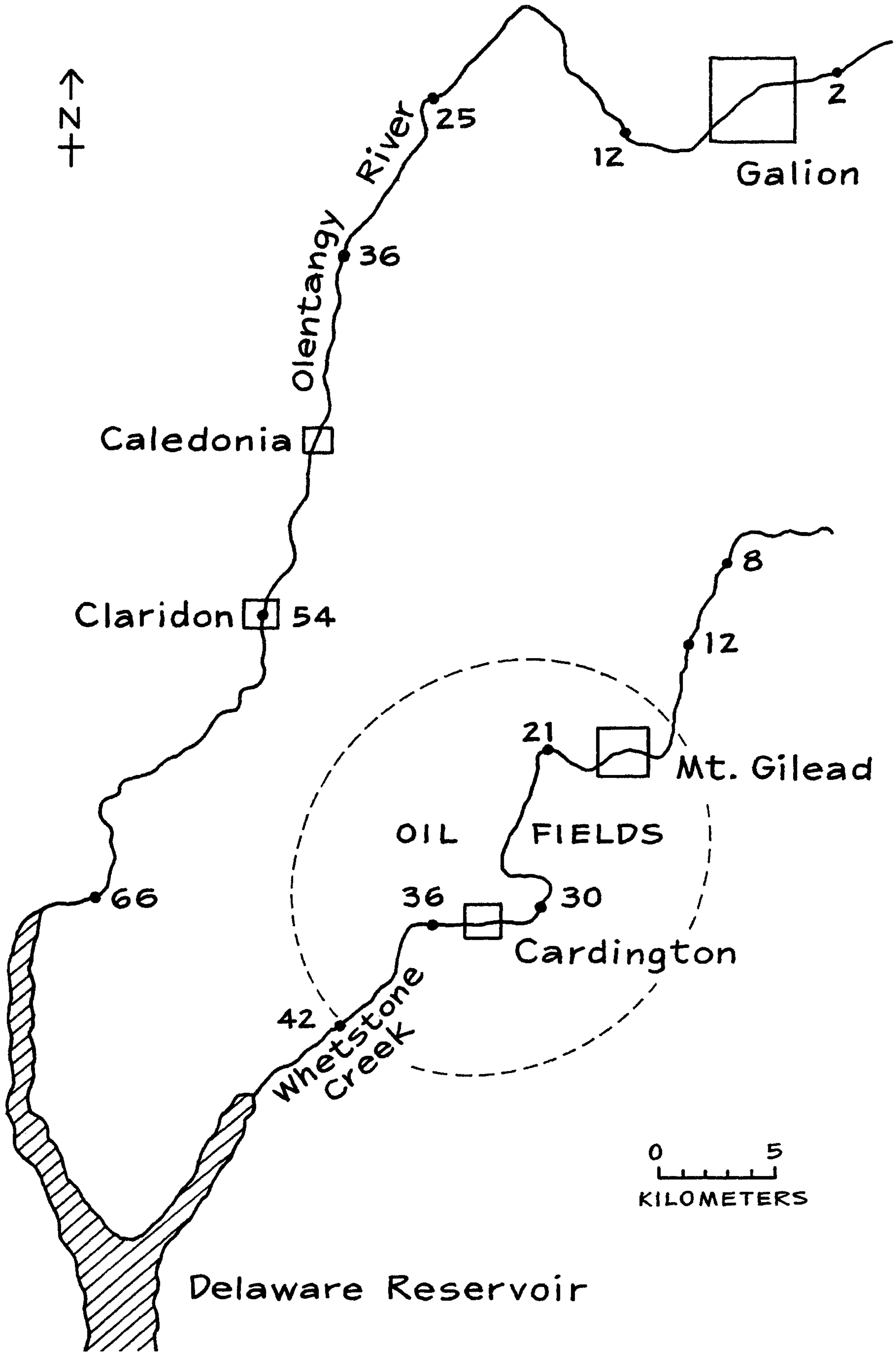

Fig. 1. Map of Whetstone Creek-upper Olentangy River study area. Sampling stations numbered in kilometers from source.

Raccoon Creek

Raccoon Creek is a permanent tributary of the Ohio River located in unglaciated southeastern Ohio (Figures 2, 3, 4). The stream originates in southern Hocking County flowing southward through Vinton and Gallia Counties to its confluence with the Ohio near Gallipolis. The main channel is 176 km long, draining approximately 1765 square km of land. The watershed is rugged, well forested and sparsely populated making this an ideal recreational area. Agriculture is restricted mostly to flood plains in downstream areas near the Ohio River. Coal, oil, gas, and lumber are the principal resources. Iron ore also is abundant, but at present is not mined. However, during the Civil War numerous blast furnaces operated in headwater regions.

Soils in the watershed are derived from parental sandstone and shales of the Mississippian and Pennsylvanian periods Muskingum silt loam is the most common soil type, but Meigs silty clay loam occurs in limited areas in the lower watershed. The soils are moderately acid with very low organic, phosphate, nitrogen and potassium content, making them generally poor for agricultural use (Roach and Pelton, 1950; Cole, 1951).

Pollution

Olentangy-Whetstone

Organic pollution is moderate in Whetstone Creek and moderate-heavy in the Olentangy River. Effluent from the sewage treatment plant at Galion enters

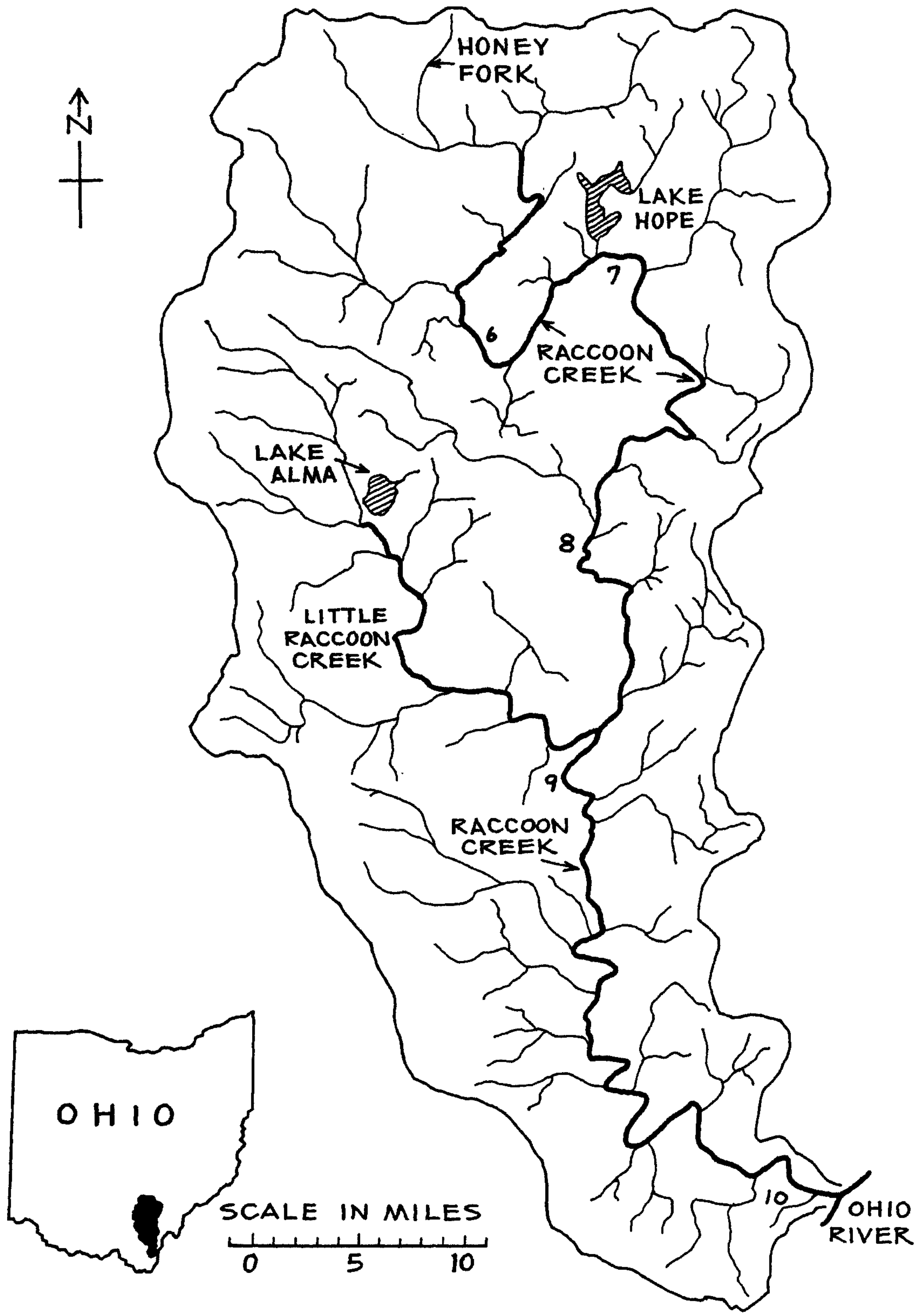

Fig. 2 Raccoon Creek drainage system. Numbers indicate location of sampling areas.

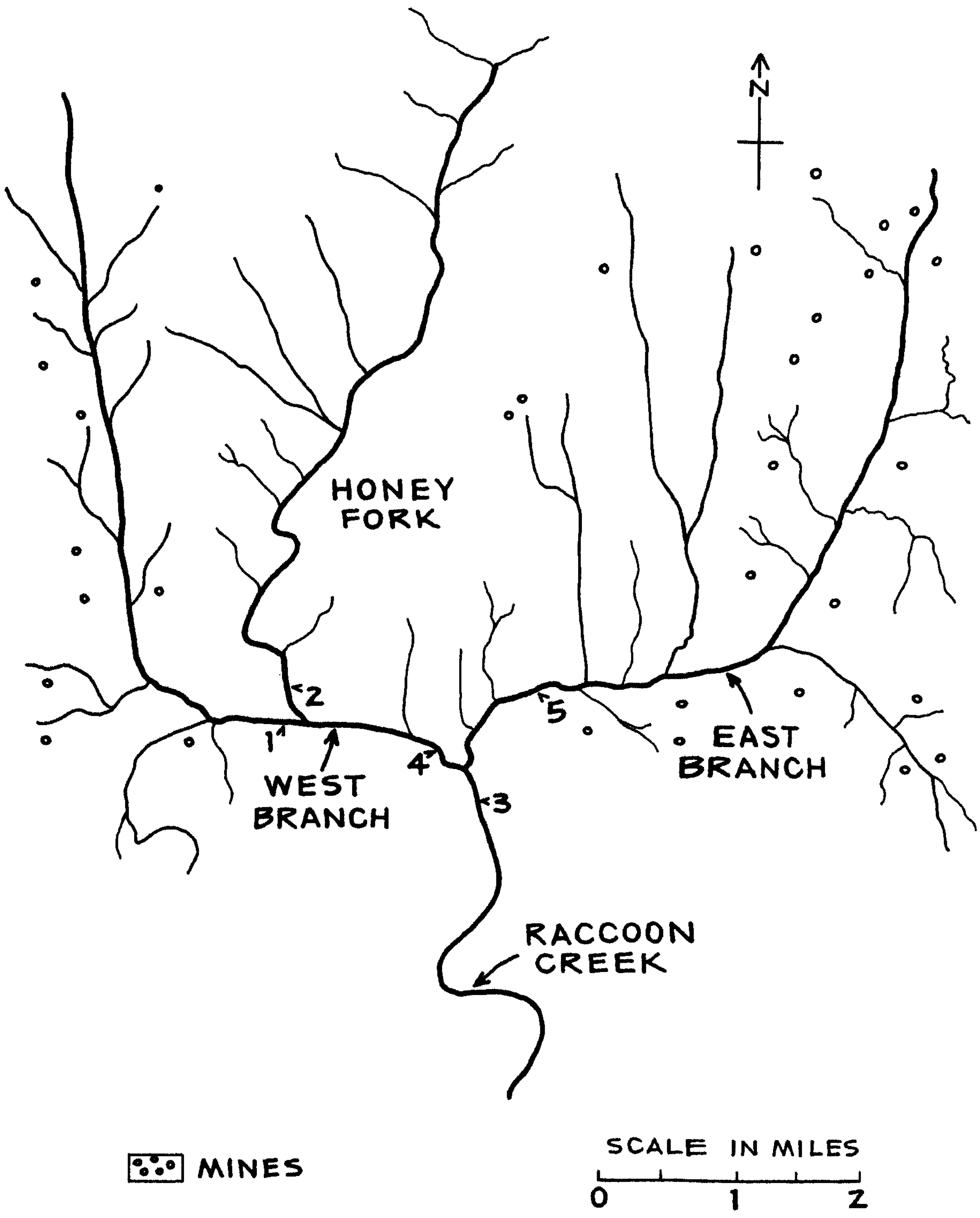

Fig. 3. Honey Fork and East-west Branch of the Raccoon Creek drainage system. Numbers indicate location of sampling areas.

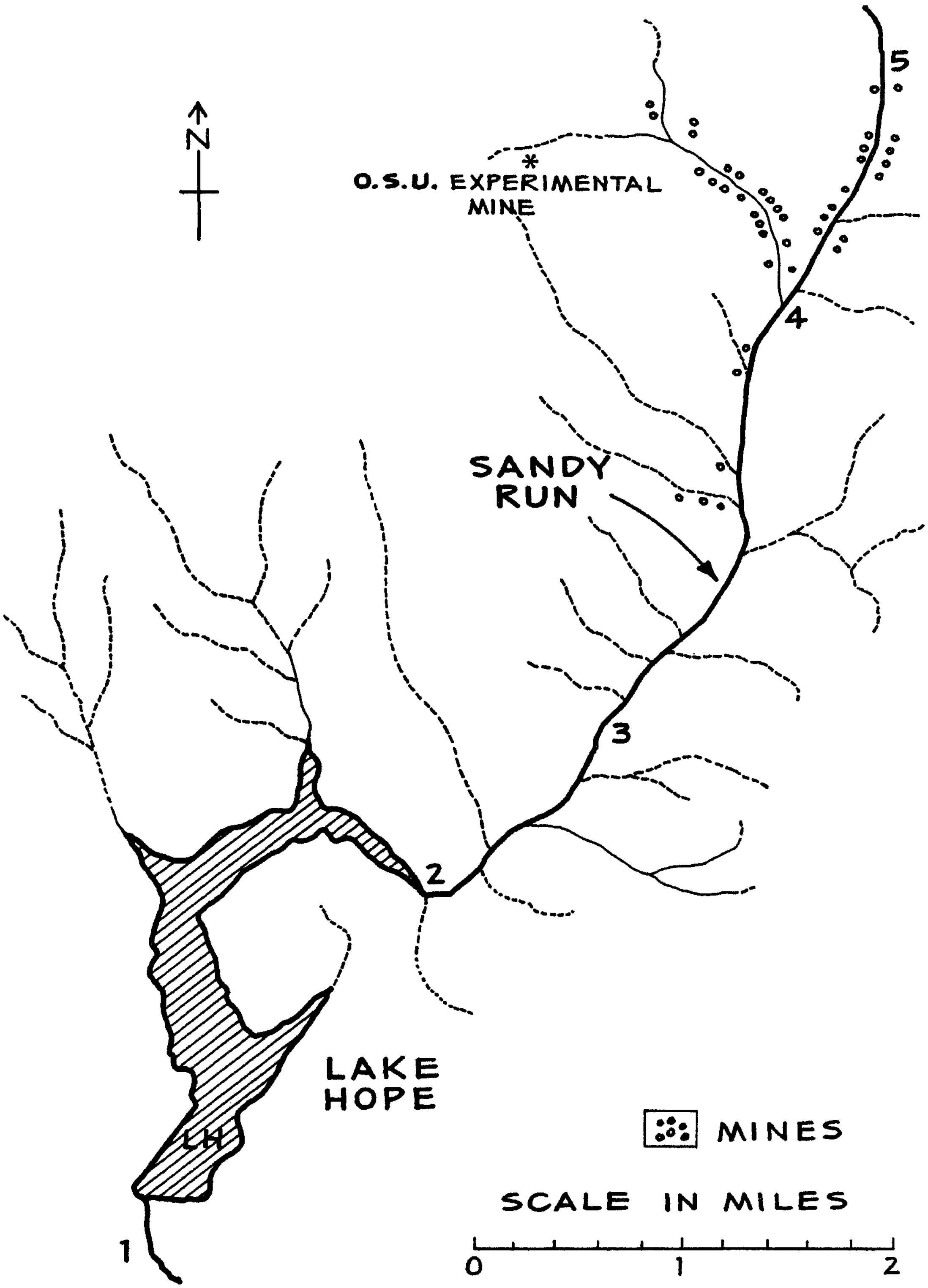

Fig. 4 Sandy Run-Lake Hope drainage system. Numbers and LH indicate location of sampling areas.

the Olentangy several kilometers below the city. This is reflected by a sharp increase in phosphates and decrease in dissolved oxygen (D.O.) at station 12 located three km below the sewage facility (Fig. 5 and 6). A gradual dilution of phosphate and increase in D.O. was noted downstream to the mouth of the Delaware reservoir. Organic materials accumulate extensively in the Olentangy because of the low stream gradient (2.8 m/km). The Galion sewage plant is a sludge-type treatment system handling an average of 182,000 m^3 per month (1966), although some raw sewage had to be by-passed a total of 111 days in 1966. Five day BOD's below the outfall ranged from 4.0 ppm in August to 8.0 ppm in September averaging 6.2 ppm for the year (Annual Summary of Operations for Sewage Treatment Plant at Galion, 1966).

Two smaller villages on the Olentangy River, Claridon and Caledonia, have no sewage systems, but most dwellings are equipped with either cess pools or septic tanks. Effluents from these facilities probably seep into the stream.

Mt. Gilead located on Whetstone Creek also has a sludge-type sewage treatment plant, handling an average of 40,000 m^3 per month. This facility did not by-pass raw sewage in 1966. Five day BOD's below the outfall ranged from 7.3 to 7.9 ppm, averaging 7.5 ppm for the year (Annual Summary of Operations for Sewage Treatment Plant at Mt. Gilead, 1966). The village of Cardington (population = 1650) has no sewage treatment system, but homes are equipped with cess pools and septic tanks.

Effluent from the Mt. Gilead sewage plant enters Whetstone Creek below the village where increased phosphate and decreased D.O. concentrations

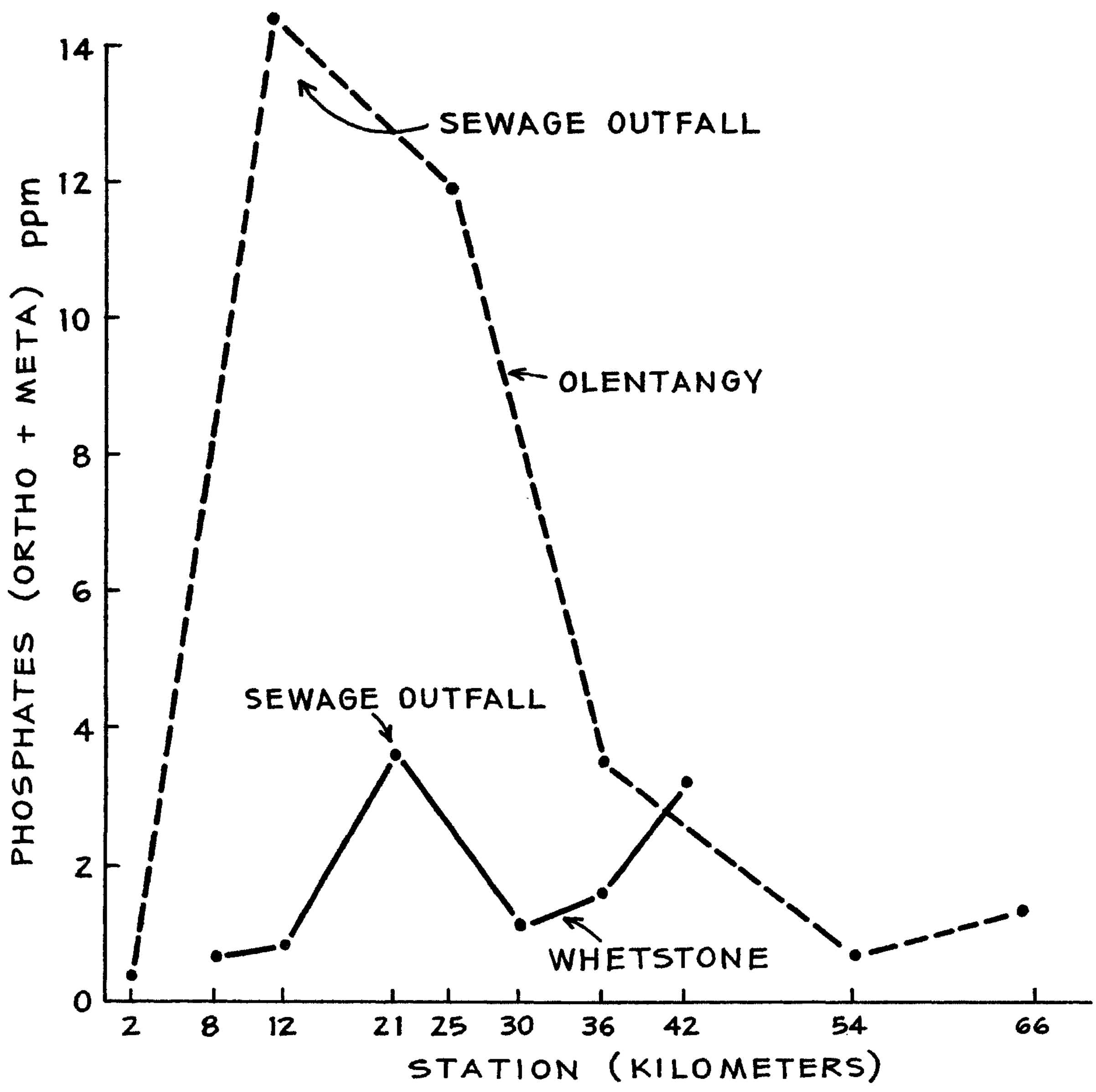

Fig. 5. Comparison of phosphate concentrations in Whetstone Creek and the upper Olentangy River. Mean values for the summers of 1965-66.

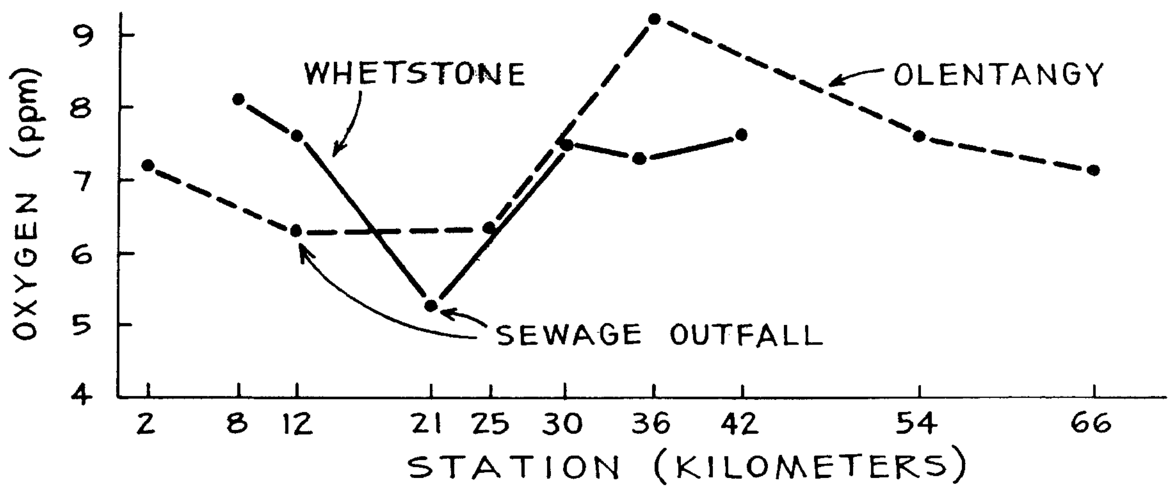

Fig. 6. Comparison of dissolved oxygen levels in Whetstone Creek and the upper Olentangy River. Mean values for the summers of 1965-66.

were noted at station 21, 2 km below the outfall (Fig. 5 and 6). Organic materials do not accumulate extensively because of the relatively steep stream gradient (4.7 m/km).

Chlorides from oil field brine wastes also are a major pollutant in Whetstone Creek. Oil drilling operations center near the middle region of the stream in the vicinity of Mt. Gilead-Cardington. Chlorides increase from less that 10 ppm above the oil fields to 200-300 ppm in downstream reaches (Fig. 7). Unfortunately, both chlorides and organic wastes enter the stream in the same region making it difficult to distinguish the effects of one or the other on benthic organisms.

Raccoon Creek

There are very few villages or individual homes near the Raccoon Creek tributaries examined in this study, and very little organic or domestic wastes enter the stream. The major pollutants are sulfuric acid and iron salts released from the oxidation of iron-sulfur compounds dispersed in the coal deposits of this region. Coal mining operations intensify the oxidation process by exposing more of the coal-iron-sulfur deposits to the air than would occur through natural erosion. The oxidation products eventually seep or flow into the streams, lowering the pH considerably and forming toxic precipitates in the stream beds. Remedial efforts are in progress in this area, but to date only experimental-scale studies have been employed.

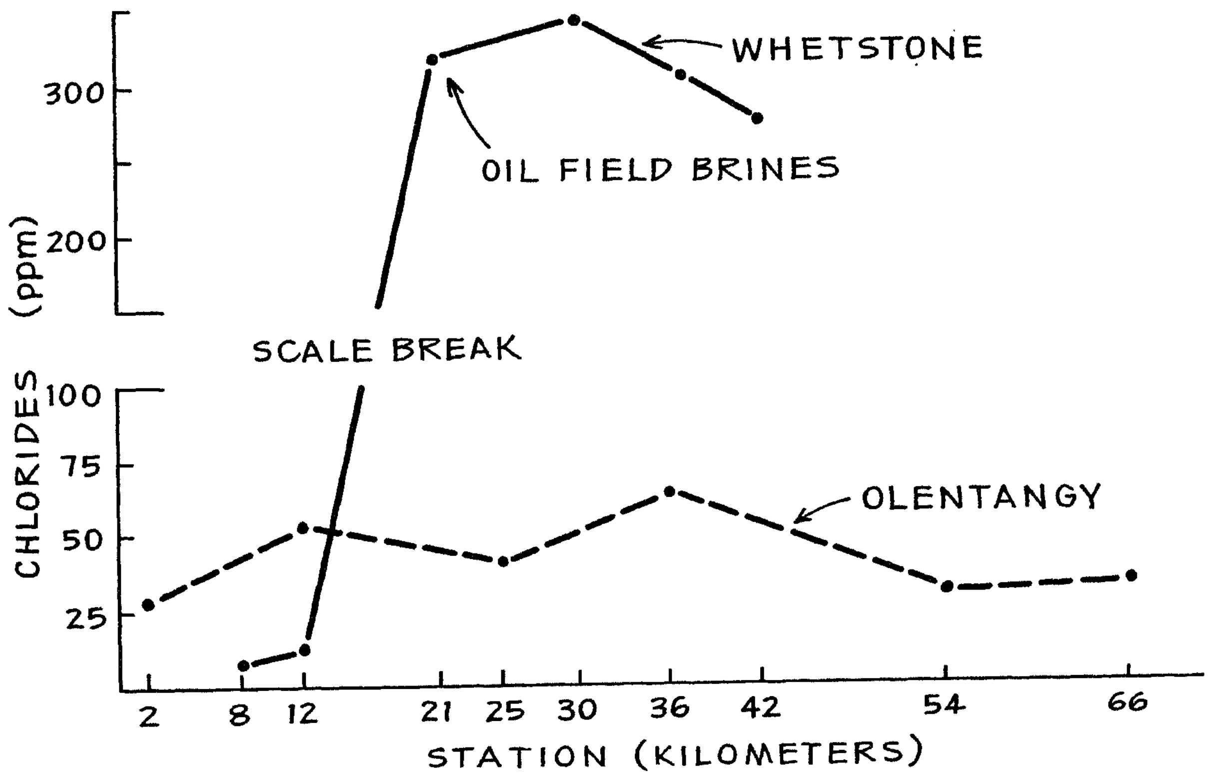

Fig. 7. Comparison of chloride concentration in Whetstone Creek and the upper Olentangy River. Mean values for the summers of 1965-66.

METHODS

Six sampling stations were established on both Whetstone Creek and the upper Olentangy River approximately five to ten kilometers apart, extending from their source near Galion to the Delaware Reservoir (Fig. 1). At Raccoon Creek, six stations were selected on the Sandy Run-Lake Hope tributary (Fig. 4), five stations in the vicinity of Honey Fork (Fig. 3), and five downstream stations on the main channel (Fig. 2). Two field crews composed of students from Ashland College, Kent State University, Ohio Wesleyan University, and The Ohio State University were trained in the procedures and worked under the direction of Dr. John H. Olive in field and laboratory situations. One student worked especially on algal organisms under the direction of Dr. C. E. Taft of The Ohio State University. Specialists were employed from time to time to make specific identifications of certain organisms and to check identifications made by field personnel.

Biological collections and physico-chemical measurements were made monthly from June to November, 1965, and from June to October, 1966 in the Whetstone Creek-Olentangy area. Sandy Run-Lake Hope and Honey Fork were sampled monthly (occasionally bi-monthly) from April, 1965 to September, 1966. Lower portions of Raccoon Creek also were examined, but collections were taken only from January to June, 1966.

Physico-Chemical

Chemical analyses including phenolpthalein alkalinity, total alkalinity, dissolved oxygen, carbon dioxide, chlorides, chromates, copper, fluoride, iron, nitrates, nitrites, phosphates (ortha and meta), silica, and sulfates were determined with a Hach water analysis kit (Hach Chemical Co., Ames, Iowa). Temperature, pH, and turbidities also were determined with this kit. Laboratory analyses by standard techniques were run from time to time to check the validity of field determinations by field kit methods. Doubtful readings were omitted from the records and calculations.

Stream flow rates were estimated by the "float method" of Robins and Crawford (1954).

Biological

Macroinvertebrates

Two 0.05 m^2 bottom samples were taken from each pool, margin, and riffle at each station. These were taken with an Ekman dredge in fine sand and mud bottoms, but by hand among coarse gravel, rocks, boulders, and leaf litter. Surber samplers were tried in some riffles but were unsatisfactory because of insufficient stream flow.

Bottom materials were placed in white enamel pans and large, easily visible organisms removed. The larger rocks, sticks, etc. were hand picked and then discarded while the finer materials were poured through a No. 40 U.S. Standard soil sieve from which the smaller benthos were removed. The macroinvertebrates then were preserved in 80 per cent ethanol containing 5 per cent glycerol, sorted by species and counted. Some specimens were forwarded to taxonomists for species identifications.

Phytoplankton and phytobenthos

Five hundred milliliter water samples were taken for quantitative phytoplankton analyses and preserved in sufficient formalin to give a five per cent solution. In the laboratory, organisms were concentrated using either centrifugation (Welch, 1948) or membrane filter techniques (McNabb, 1960). The latter technique was not useful in Raccoon Creek because of a low phytoplankton: debris ratio. Phytoplankton was enumerated using a compound microscope, a calibrated Whipple ocular and Sedgewick-Rafter cell according to the methods of Welch (1947). Grab samples of phytobenthos were taken from microhabitats at each station, but no quantitative work was done with these organisms.

Fish

Fish were collected in the Whetstone Creek-Olentangy area twice during the summer of 1965 and once in the summer of 1966. No fish collections were made in Raccoon Creek. A four by ten foot bag-type, one-quarter inch mesh minnow seine was used. Collected fishes were fixed in eight per cent formalin for two weeks, washed in water and stored in fifty per cent isopropyl alcohol.

RESULTS

Physico-Chemical

Whetstone Creek

Results are shown in Tables 1 and 2. Alkalinities were due entirely to carbonates and bicarbonates with the latter nearly always exceeding the carbonate alkalinity. One exception occurred on 25 August 1966 at station 42 where 82 ppm carbonate alkalinity and only 48 ppm bicarbonate alkalinity was recorded. This measurement was made during low stream flow and may reflect photosynthetic utilization of bicarbonates by phytoplankton and phytobenthos. High carbonates also were obtained on the same date at Station 8, although carbonates did not exceed bicarbonates at this station. In general, alkalinities were "moderate" compared to most fresh water streams.

Chlorides were low at headwater stations 8 and 12, but increased considerably at all sites downstream from the oil fields. Brines containing large quantities of chlorides overflow from oil drilling operations in this area and, although some protective measures are taken, considerable amounts seep into the stream.

Chromates, copper, fluoride, iron, and manganese were present in trace quantities and seldom exceeded 1 ppm. Very little variation was noted at any station except for manganese which tended to be more highly concentrated at station 42 during low flows.

The major nutrients, nitrates, phosphates, and silicates varied widely

Table 1. Summary of physico-chemical data, Whetstone Creek. Means of 2-3 samples taken between 1-June and 30-August, 1966.

	Station (km from source)						
	8	12	21	30	36	42	$\bar{X}$
Alkalinity-Phenol-phthalein**	21	12	11	10	12	27	15.5
Alkalinity-Total**	221	244	230	239	262	204	233.0
Carbon Dioxide	15	19	22	19	41	19	22.5
Chloride	7	10	232	187	261	220	153.0
Hardness-Ca**	165	200	248	262	265	276	236
Hardness-Mg**	65	97	116	142	133	105	110
Iron	0.3	0.3	0.2	0.2	0.2	1.1	0.4
Manganese	0.4	0.3	0.8	0.6	0.5	1.3	0.6
Nitrate	1.4	1.36	1.78	-	1.2	0.96	1.3
Dissolved Oxygen	8.1	8.2	5.3	6.7	7.5	7.3	7.2
Phosphate-Ortho	0.38	.1	2.2	1.05	1.0	3.26	1.3
Phosphate-Meta	0.27	.41	0.4	-	.61	2.46	0.8
Silica	7.5	6.4	12	8.4	10.4	14.1	9.8
Sulfate	80	73	94	90	102	111.0	91.6
pH	8	8	7.6	8.1	7.4	8.3	7.9
Turbidity-(ppm, Formazin Std., Hach Field Kit)	34	24	18	18	20	43	24.5
Temperature (°C)	17	17	18.7	-	25	25	20.5
Flow rate (m^3/sec.)	0.06	0.13	0.39	0.26	0.82	0.57	0.40

*All analyses given in ppm of indicated material except as noted.
**ppm as $CaCO_3$.

Table 2. Summary of physico-chemical data, Whetstone Creek, June - September 1965.*

	Station					
	12	21	30	36	42	$\bar{X}$
Alkalinity-Phenolphthalein**	22	16	24	15	28	21
Alkalinity-Total**	250	262	240	215	168	227
Carbon Dioxide	-0-	-0-	-0-	-0-	-0-	-0-
Chloride	12	405	505	350	323	319
Hardness-Ca**	212	290	-	260	270	258
Hardness-Mg**	105	210	-	170	140	156
Iron	0.4	0.3	0.1	0.2	0.2	0.2
Manganese	-0-	-	-	-	-	
Nitrate	-	-	-	1.8	1.6	1.7
Dissolved Oxygen	7.0	-	8.5	7.2	8.0	7.7
Phosphate-Ortho	0.7	4.1	1.2	1.2	0.5	1.5
Phosphate-Meta	0.5	0.6	-0-	0.4	0.2	0.3
Silica	47.0	79.0	33	8	8	35.
Sulfate	-	-	-	-	-	
pH	6.8	8.5	8.4	8.5	8.4	8.1
Turbidity-(ppm, Formazin Std., Hach Field Kit)	28	23.0	28	21	19	23.8
Temperature (°C)	22	23.0	-	-	-	22.5
Flow rate (m^3/sec.)	0.13	.25	0.22	0.28	0.25	0.23

*All analyses given in ppm of indicated material except as noted.
**ppm as $CaCO_3$.

from date to date. No significant variation in nitrates was noted between stations, however phosphates were much higher at all stations below the Mt. Gilead sewage outfall than at upstream reaches. Silica varied considerably from station to station and no consistent pattern emerged.

Dissolved oxygen averaged 7.4 ppm ranging from 4.3-9.5 ppm. Lowest values occurred at station 21 below the Mt. Gilead sewage outfall, but a slight increase of 1-2 ppm was noted further downstream. Upstream stations maintained the highest oxygen levels. No unusual patterns of concentration were noted for carbon dioxide, sulfates, calcium, magnesium, pH, or turbidity.

Olentangy River

Results are shown in Tables 3 and 4. Bicarbonates and carbonates accounted for the total alkalinity at all stations except station 12 (Galion sewage outfall) where 40 ppm alkalinity was due to hydroxides during low flow in the fall of 1965. No concentration patterns were noted along the length of the stream, but wide variations occurred from one sampling period to another at the same station. For example, total alkalinity at station 66 ranged from 150 ppm on July 19, to 500 ppm on August 29, 1966. Chromates, copper, fluorides, iron, and manganese seldom exceeded 1 ppm and were not concentrated at any particular station. The major nutrients, nitrates, phosphates, and silicates varied from station to station and from date to date. In 1966, nitrates were highest in lower reaches of the stream, but in 1965 upstream stations contained the highest nitrate levels. Phosphates were significantly higher at station 12 near the sewage outfall, but were gradually diluted to less than 1 ppm at downstream stations. Silica appeared to be slightly higher

Table 3. Summary of physico-chemical data, Upper Olentangy River. Mean of 2-3 samples taken between 1 June and 30 August 1966.*

	Station (km from source)						
	2	12	25	36	54	66	$\bar{X}$
Alkalinity-Phenol-phthalein**	12	10	12	8	17	6	10.8
Alkalinity-Total**	159	240	216	190	160	287	209
Carbon Dioxide	12	19	14	13	8	16	13.6
Chloride	28	40	37	39	27	29	33
Hardness-Ca**	122	165	159	189	192	225	175
Hardness-Mg**	103	120	96	107	75	45	91
Iron	0.7	0.3	0.4	0.4	0.2	0.4	0.4
Manganese	0.4	1.7	0.4	0.8	0.5	0.6	0.7
Nitrate	0.43	.35	0.5	1.53	3.35	3.82	1.7
Dissolved Oxygen	7.2	4.9	6.4	6.6	8.9	6.1	6.7
Phosphate-Ortho	.44	10.2	5.0	2.3	0.4	0.24	3.1
Phosphate-Meta	.27	3.06	5.1	.25	0.5	0.27	1.6
Silica	35.2	7.4	8.6	10.1	4.2	5.4	11.8
Sulfate	95.0	125	115.	116.6	180	88.3	120.0
pH	7.8	8.0	8.1	8.3	7.9	8.0	8.0
Turbidity-(ppm, Formazin Std., Hach Field Kit)	17	17	36	52	85	93	50.0
Temperature (^{o}C)	23	22	24	24	23	23	23.5
Flow rate (m^3/sec.)	0.02	0.14	0.22	0.71	3.3	1.4	0.96

*All analyses given in ppm of indicated material except as noted.
**ppm as $CaCO_3$.

Table 4. Summary of physico-chemical data, Upper Olentangy River, September - October 1965.*

	Station 12	25	36	54	66	$\bar{X}$
Alkalinity-Phenol-phthalein**	70	22	35	40	20	37.4
Alkalinity-Total**	100	170	225	180	190	173.0
Carbon Dioxide	12	6	4	6	6	6.8
Chloride	67	48	89	35	37	55.2
Hardness-Ca**	120	150	136	152	145	140.6
Hardness-Mg**	50	50	87	77	95	71.8
Iron	0.4	0.3	0.2	0.1	0.1	.2
Manganese	-	-	-	-	-	
Nitrate	1.4	7.9	3.2	-0-	-0-	2.5
Dissolved Oxygen	7.7	6.2	11.8	6.3	8.1	8.0
Phosphate-Ortho	13.5	9.5	3.8	0.4	1.2	5.7
Phosphate-Meta	2.7	4.2	0.8	0.2	0.9	1.7
Silica	9	5	1.7	4	0.8	4.1
Sulfate	-	-	-	-	-	
pH	7.8	7.7	8.1	8.0	8.4	8.0
Turbidity-(ppm Formazin Std., Hach Field Kit)	30.	17	20	15	14	19.2
Temperature (°C)	12	12	12	12.5	11.5	12.0
Flow rate (m^3/sec.)	.09	0.14	0.19	0.26	0.29	0.19

*All analyses given in ppm of indicated material except as noted.
**ppm as $CaCO_3$.

in upstream reaches, but no clear cut patterns of distribution were noted.

Dissolved oxygen was lowest and carbon dioxide highest at station 12 but farther downstream this situation was gradually reversed. No unusual patterns of concentration were noted for chlorides, calcium, magnesium, sulfates, pH, or turbidity.

Sandy Run-Lake Hope

Results of physico-chemical analyses are shown in Table 5. In general, Sandy Run-Lake Hope water is characterized by low total alkalinities, low pH, and high iron, manganese, and sulfates. Most other measurements were near "normal" for Ohio surface water. Alkalinities (and pH), although low, were highest at unpolluted station 5, dropping markedly to 0 ppm at polluted stations 3 and 4, but recovering slightly (10 ppm) in Lake Hope and station 1 below the lake. Iron, manganese, sulfate, calcium, magnesium, mineral acidity, carbon dioxide, and silica were highest at stations 3 and 4 nearest the acid-mine outfalls but near "normal" values were encountered in Lake Hope and at stations 1 and 5. Some values obtained for Ca, Mg, and Si may be erroneously high because of iron interference. All stations were well oxygenated, averaging over 8.0 ppm dissolved oxygen, and no measurement less than 5.3 ppm was recorded. Phosphates and nitrates were low at all stations, seldom exceeding 0.5 ppm.

Honey Fork and Vicinity

A summary of physical-chemical data for unpolluted (station 2) vs polluted (stations 1, 3, 4, 5) in the Honey Fork area is shown in Table 6. Unpolluted station

Table 5. Summary of physico-chemical data, Sandy Run - Lake Hope. Annual means, 1965-66.*

	Station (← Direction of Flow)				
	1	LH_S	3	4	5
Acidity-CO_2**	10.4	11	78	418.5	8.2
Acidity-Mineral**	0.4	0.7	19.9	383.7	0
Alkalinity-Phenol-phthalein**	0	0	0	0	0
Alkalinity-Total**	10	7	0	12	54.8
Chloride	12	12	11.4	3.3	19.6
Hardness-Ca**	39	38	124	231	67
Hardness-Mg**	32	36	119	225	41
Iron	0.5	0.5	4.9	28.2	0.2
Manganese	1.6	2.3	6.7	11.	0.4
Nitrate	0.38	0.15	0.03	0.4	0.8
Dissolved Oxygen	10.3	10.5	9.2	8.2	11.1
Phosphate-Ortho	0.2	0.1	.09	0.09	0.1
Phosphate-Meta	0.12	0.2	0.3	0.36	0.2
Silica	61	77	199	346	88
Sulfate	72	84	337	1126	42
pH	4.4	4.0	3.5	2.9	5.1
Turbidity-(ppm Formazin Std., Hach Field Kit)	8.4	4.4	9.3	25.8	8.2
Temperature (^{o}C)	15.8	15.8	13.6	13.2	13.7

*All analyses given in ppm of indicated material except as noted.
**ppm as $CaCO_3$.

two contains a higher total alkalinity, turbidity, and pH, while polluted stations maintain higher CO_2, mineral acidity, Ca, Mg, Fe, Mn, Si, and sulfates. Other resources including chlorides, nitrates, phosphates, and oxygen appear evenly distributed at all stations. All sites were well oxygenated, averaging over 8 ppm. Only two of thirty-six oxygen determinations made in the Honey Fork area were less than 5 ppm.

Table 6. Summary of physico-chemical data, Honey Fork Area (Raccoon Creek). Annual means, 1965-66.*

Station -	W.Br. 1	Honey Fk. 2	Raccoon Cr. 3	W. Br. 4	E. Br. 5
Acidity-CO_2**	218	8	163	55	307
Acidity-Mineral**	46	0	20	9.7	47
Alkalinity-Phenolphthalein**	0	0	0	0	0
Alkalinity-Total**	0	54	1	3.3	0
Chloride	16	36	13	35	5
Hardness-Ca**	157	56	150	74	280
Hardness-Mg**	133	40	229	85	404
Iron	6.7	0.9	7.5	2.4	12.5
Manganese	11.3	1.9	13.7	6.5	27.1
Nitrate	0.49	0.26	0.14	0.13	0.26
Dissolved Oxygen	8.9	9.5	9.7	9.6	10.1
Phosphate-Ortho	0.15	0.17	0.14	.06	0.12
Phosphate-Meta	0.32	0.21	0.23	.23	0.31
Silica	266	40	148	130	355
Sulfate	496	37	675	202	1106
pH	3.3	5.6	3.4	3.9	3.2
Turbidity-(ppm Formazin Std., Hach Field Kit)	12.2	25.7	22	12	11.5
Temperature (°C)	13.2	14.1	12.9	13.2	13

*All analyses given in ppm of indicated material except as noted.
**ppm as $CaCO_3$.

Algae

Whetstone Creek

The algal flora collected at each station is shown in Table 7. No attempt was made to separate phytobenthos from phytoplankton because in headwater streams many organisms such as the diatoms begin growth as benthic flora, become detached later and float free as part of the phytoplankton. This is probably not true of larger rivers where the potamoplankton forms an important part of the total flora (Williams, 1964).

Diatoms and green algae accounted for 36 of the 43 genera of algae collected in Whetstone Creek. The greatest diversity of genera of diatoms was found at headwater station 8, although a large diversity was noted at stations 21 and 42. All genera collected at station 8 also were found downstream except Anomoeneis, Cocconeis, Diploneis, Gomphoneis, Nitzschia, and Tabellaria. Diploneis was found as far downstream as station 21. Only two genera, Stauroneis and Surirella, occurred at downstream stations 36 or 42, but never in headwater stations.

Cyclotella, Cymbella, Gomphonema, Gyrosigma, Navicula, Rhoiocosphenia and Synedra were found at four of the six stations. Pinnularia occurred at station 21, the site nearest the Mt. Gilead sewage outfall.

Green algae were most abundant in the lower half of Whetstone Creek from station 21 to the Delaware Reservoir. A wider diversity of genera also occurred in this area. Only two genera, Closterium and Rhizoclonium, appeared at

Table 7. Phytoplankton and phytobenthos, Whetstone Creek, 1 June - 1 October 1966. *Asterik indicates at least one occurrence.

	Station					
	8	12	21	30	36	42
Bacillariophyceae						
Anomoeoneis	*					
Caloneis	*					*
Cocconeis	*					
Cyclotella	*	*	*	*	*	*
Cylindrotheca	*					*
Cymbella	*	*	*			*
Diploneis	*		*			
Fragilaria	*		*			*
Gomphoneis	*					
Gomphonema	*		*	*	*	
Gyrosigma	*	*	*		*	*
Melosira	*		*			*
Navicula	*	*	*	*	*	*
Nitzschia	*					
Opephora	*		*			*
Pinnularia			*			

Table 7. Cont'd.

	Station					
	8	12	21	30	36	42
Rhoiocosphenia	*	*	*	*		*
Stauroneis						*
Stephanodiscus	*		*		*	
Surirella					*	*
Synedra	*	*	*			*
Tabellaria	*					
Chloromonadophyta						
Gonyostomum			*			
Chlorophyceae						
Ankistrodesmus					*	
Chlamydomonas			*		*	*
Cladophora			*			*
Closterium	*	*	*	*	*	
Cosmarium	*		*			
Dysmorphococcus			*			
Eudorina					*	

Table 7. Cont'd.

	Station					
	8	12	21	30	36	42
Pandorina			*		*	
Pediastrum			*		*	*
Rhizoclonium	*	*	*	*		
Scenedesmus					*	*
Spirogyra			*			*
Chrysophyceae						
Synura					*	
Cyanophyceae						
Oscillatoria	*		*	*	*	*
Euglenaceae						
Euglena			*		*	*
Phacus			*		*	*
Trachelomonas					*	

four or more of the station. Cosmarium was found only in headwater areas and Dysmorphococcus occurred only at station 21. Scenedesmus and Eudorina were restricted to pools at the extreme downstream stations 36 and 42.

Euglenoids and the Chrysophyte, Synura appeared only in pools in the lower portions of the stream. Oscillatoria was the only blue-green alga observed, appearing at all stations except headwater No. 12.

Olentangy

The algal flora collected at each station is shown in Table 8. Diatoms and green algae accounted for 39 of 49 genera collected in the Olentangy River. The greatest diversity of diatoms (15-18 genera) occurred at headwater stations 2, 12, and 25 although 12 genera were found at station 66. Cyclotella, Cylindrotheca, Diploneis, Fragilaria, Gyrosigma, Navicula, Nitzschia, Rhoiocosphenia, Surirella, and Synedra were collected at 4 of the 6 stations. Caloneis, Gomphoneis, Melosira, Meridon and Tabellaria occurred only at upstream stations. No diatom was found at downstream stations 54 or 66 that did not occur further upstream. Diatoma occurred only at station 25, Melosira and Meridion at station 12, and Caloneis at station 2.

Green algae were evenly distributed at each station ranging from five genera at station 66 to ten genera at station 12 (sewage outfall). Closteriopsis, Closterium, Pediastrum, and Scenedesmus appeared at 4 or more of the 6 stations. Hydrodictyon, and Tetrastrum occurred only at station 12, Micrasterias at station 54, and Staurastrum at station 66. Cosmarium, Cladophora, Hydrodictyon, and

Table 8. Phytoplankton and phytobenthos, Upper Olentangy River, 1 June - 1 October, 1966. Asterisk (*) indicates at least one occurrence.

	Station					
	2	12	25	36	54	66
Bacillariophyceae						
Anomoeneis						
Caloneis	*					
Cocconeis						
Cyclotella	*	*	*	*		*
Cylindrotheca	*	*		*		*
Cymatopleura		*			*	
Cymbella	*	*				
Diatoma			*			
Diploneis	*	*	*	*		
Fragilaria	*	*	*		*	*
Gomphoneis	*	*				
Gomphonema	*	*				*
Gyrosigma	*		*	*	*	*
Melosira		*				
Meridion		*				
Navicula	*	*	*	*	*	*
Nitzschia	*	*	*			*
Opephora		*	*			*
Pinnularia			*			*

Table 8. Cont'd.

	Station					
	2	12	25	36	54	66
Rhoiocosphenia	*	*	*			*
Stauroneis	*		*			
Stephanodiscus		*	*	*		
Surirella	*	*	*	*	*	*
Synedra	*	*	*	*	*	*
Tabellaria		*	*			
Chloromonadophyta						
Gonyostomum				*		
Chlorophyceae						
Actinastrum		*	*	*		
Ankistrodesmus		*	*	*		
Chlamydomonas			*			
Cladophora	*	*				
Closteriopsis			*	*	*	*
Closterium	*	*	*		*	*
Cosmarium	*	*				
Hydrodictyon		*				

Table 8. Cont'd.

	Station					
	2	12	25	36	54	66
Micrasterias					*	
Pandorina	*			*		
Pediastrum	*	*	*	*	*	*
Pteromonas		*		*	*	
Rhizoclonium	*				*	
Scenedesmus	*	*	*	*	*	*
Staurastrum						*
Tetrastrum		*				
Chrysophyceae						
Synura	*	*				*
Cyanophyceae						
Merismopedia	*	*	*	*		
Oscillatoria	*	*	*	*	*	*
Anabaena	*					
Euglenaceae						
Euglena	*	*	*	*		*
Phacus	*	*	*	*	*	*

Table 8. Cont'd.

	Station					
	2	12	25	36	54	66
Pyrrophyta						
Ceratium			*	*		*
Gymnodinium			*			
Xanthophyceae						
Arachnochloris			*		*	

Tetrastrum were restricted to headwater areas.

The Cyanophyceans, Merismopedia and Oscillatoria occurred at most stations as did the euglenoids, Euglena and Phacus. Ceratium, Gymnodinium and Arachnochloris appeared only at downstream stations 25-66.

Gyrosigma, Stauroneis, Pandorina, and Rhizoclonium were absent at station 12 (sewage outfall), but present both upstream and downstream from this station.

Sandy Run-Lake Hope

The annual mean numbers of algae per ml and their frequency of occurrence are shown in Table 9. Green algae accounted for 52 per cent, diatoms 24 per cent, and euglenoids 13 per cent of the 46 genera collected. Of these, only Eunotia, Navicula, Synedra, and Trachelomonas were collected from each station at least once during the annual cycle. Closterium acutum, Staurastrum, Surirella Euglena mutabilis, Phacus, and Peridinium inconspicuum were collected at three or more of the six stations.

The greatest diversity of genera occurred at unpolluted headwater station 5 and station 1, the outflow from Lake Hope. Diversity of genera was lowest in Lake Hope, however, more than ten times as many organisms per ml occurred in the Lake as in the "stream" stations of Sandy Run.

C. acutum usually accounted for more than 80 per cent (700/ml) of the organisms in Lake Hope and was present in 75 per cent of the samples. P. inconspicuum also was present in over 75 per cent of the samples but usually accounted

Table 9. Phytoplankton and phytobenthos, Sandy Run - Lake Hope. Frequency of occurrence (F) and annual mean ($\overline{X}$) numbers of organisms per ml. LH_S, LH_2, LH_4, LH_6 = Lake Hope surface, 2, 4, and 6 meter depths. * = less than one (1).

	Station													
	1	LH_S		LH_2		LH_4		LH_6		2		3	4	5
Organism	F	F	$\overline{X}$	F	$\overline{X}$	F	$\overline{X}$	F	$\overline{X}$	F	$\overline{X}$	F	F	F
Chlorophyta														
Chlamydomonas	42									7	*	41	22	27
Chlorella												3	2	
Cladophora	3													11
Closteriopsis	8	2	*					12	*				1	27
Closterium sp. A	67							4	*			19		
Closterium acutum		68	885	73	695	71	733	73	1043	50	9	31		
Closterium sp. B										39	3		12	70
Coccomyxa	3													
Cosmarium	3			2	*					7	*	3	2	21
Euastrum	6			2	*									
Microspora	6											39	7	5
Mougeotia	22											11	7	54
Oedogonium												3		
Pandorina														3
Rhizoclonium	6											3		
Scenedesmus	6	2	*	2	*									
Selenastrum	6	5	*	4	*	5	*							
Spirogyra														14
Staurastrum	53	20	*	10	*	18	*	23	*	21	*	4		3
Stigeoclonium												7		24
Ulothrix	78	2	*											
Ulothrichales												46	12	3
Zygnema	3													
Chrysophyta														
Cymbella	6					2	*					3	3	86
Eunotia	83	60	3	46	2	58	3	42	3	75	6	93	76	8
Gomphonema												3	3	51
Gyrosigma														33
Melosira												4	1	59
Meridion										4	*	4	2	22

Table 9. Cont'd.

Organism	Station 1	LH_S		LH_2		LH_4		LH_6		2		3	4	5
	F	F	$\overline{X}$	F	$\overline{X}$	F	$\overline{X}$	F	$\overline{X}$	F	$\overline{X}$	F	F	F
Navicula	94	80	5	73	5	82	8	92	10	50	2	79	48	84
Pinnularia	14									4	*	3		19
Rhopalodia	8													16
Surirella		2	*	2	*	2	*	8	*			1		27
Synedra	33	7	*	5	*	2	*	4	*	7	*	6	3	92
Cyanophyta														
Anabaena	28													27
Microcystis														3
Oscillatoria	14												1	24
Euglenophyta														
Euglena mutabilis	19	32	10	41	5	49	9	69	35	46	2	40	30	
Euglena sp.		2	1	2	*	5	*						4	
Euglenoid												9	1	14
Eutreptia	22													
Phacus	11	7	*	14	*	22	*	38	3	7	*			
Trachelomonas	19	18	*	18	*	24	*	27	*	36	2	1	3	5
Pyrrhophyta														
Peridinium inconspicuum	14	75	176	79	149	76	118	77	126	57	53	4	1	
Others														
Phytoflagellate	25											23	5	30

for less than 15 per cent (150/ml) of the organisms. Eunotia and Navicula appeared in over 50 per cent of the samples, but averaged less than ten organisms per ml. Chlamydomonas, Cladophora, Cosmarium, Mougeotia, Melosira, Pinnularia, Rhopalodia, Anabaena, and Oscillatoria appeared at both unpolluted station 5 and station 1 below the Lake Hope outflow, but seldom at polluted stations between or in Lake Hope. When these genera were noted at polluted stations 2, 3, or 4, their presence could be attributed to drift from station 5. Special note should be made that blue-green algae occurred only at unpolluted station 5 and station 1 and were never found in the highly polluted reaches or in Lake Hope.

Two organisms previously unreported in acid-mine water were Peridinium inconspicuun Lemm. and Closterium acutum (Lyn.gb) Bréb. These were dominant organisms in Lake Hope.

Honey Fork and Vicinity

The annual mean members of algae per ml and their frequency of occurrence are shown in Table 10. Green algae accounted for 37 per cent, diatoms 31 per cent, euglenoids 14 per cent, and blue-greens 11 per cent of the 35 genera collected. The largest diversity of genera (30) occurred at Honey Fork station 2, the only unpolluted tributary in the study area. West Branch station 4 located several kilometers downstream, also contained a wide diversity of genera (19), probably because of inflow from unpolluted Honey Fork station 2. Other stations in the area, all receiving acid-mine drainage, contained fewer than 12 genera. Blue-

Table 10. Phytoplankton and phytobenthos, Honey Fork and vicinity, Raccoon Creek. Frequency of occurrence (F) and annual mean ($\overline{X}$) numbers o organisms per ml. * = less than 1.

Organism	West Br. 1 F	West Br. 1 $\overline{X}$	Honey Fk. 2 F	Honey Fk. 2 $\overline{X}$	Raccoon Cr. 3 F	Raccoon Cr. 3 $\overline{X}$	West Br. 4 F	West Br. 4 $\overline{X}$	East Br. 5 F	East Br. 5 $\overline{X}$
Chlorophyta										
Ankistrodesmus			25	4						
Chlamydomonas	50		13		28		33	*	33	
Chlorotylium							17			
Closteriopsis			25	*	14	*	33	*		
Closterium	67	10	38	15			33	7		
Microspora	50				14		17		33	
Mougeotia	33									
Pediastrum			13	*						
Scenedesmus			38	14						
Spirogyra			38							
Staurastrum			25	*			17			
Stigeoclonium			13							
Ulothrichales									17	
Chrysophyta										
Coscinodiscus			13							
Cymbella			25	*						
Eunotia	100	14	25	*	71	3	33	*	100	69
Gomphonema			13	*			17	*		
Gyrosigma			25	*						
Melosira			13							
Navicula	100	2	100	48	86	15	100	47	100	35
Pinnularia	17		38				33			
Surirella			25				17			
Synedra	33		88	4	14	*	17		17	17
Dinobryon			38	284						

Table 10. Cont'd.

Organism	West Br. 1		Honey Fk. 2		Raccoon Cr. 3		West Br. 4		East Br. 5	
	F	$\bar{X}$	F	$\bar{X}$	F	$\bar{X}$	F	$\bar{X}$	F	$\bar{X}$
Cyanophyta										
Dactylococcopsis			38	273						
Microcystis			13				17			
Oscillatoria			75	3			50	*		
Spirulina			13	*						
Euglenophyta										
Euglena mutabilis	100	1			43		50	8	50	
Euglena	50	*	63	70	14		17	2		
Phacus			13	*			17	1		
Strombomonas			13							
Trachelomonas			38	354	14		50	2	33	*
Pyrrophyta										
Dinoflagellate	33	2	38	5	29	1			50	4
Others										
Phyto-flagellate	33	518	38	*	14		17		17	

green algae appeared only at Honey Fork station 2 and West Branch station 4, occurring more frequently and in greater numbers at the former site.

Eunotia, *Navicula*, and *Synedra* were the only genera found at all stations. *Chlamydomonas*, *Microspora*, *Eunotia*, and *E. Mutabilis* appeared more frequently in polluted areas than at unpolluted Honey Fork station 2.

Unidentified phytoflagellates, *Eunotia*, and *Navicula* accounted for the largest number of organisms/ml at polluted stations, while *Dinobryon*, *Dactylococcopsis*, *Euglena* and *Trachelomonas* accounted for most organisms at station 2.

Benthic Macroinvertebrates

Whetstone Creek

The composition of the benthic macroinvertebrate community is shown in Tables 11 and 12. Based upon averages of all stations in the summer, 1966, molluscs were the most abundant group of organisms at the first four headwater stations 8, 12, 21, and 30 accounting for 83.8 per cent of the community at station 12, but only 1 per cent at station 42. Trichopterans made up 27 per cent of the community at station 8, declining gradually downstream to 0 per cent of the total at station 42. In contrast, Oligochaetes increased progressively from 0 per cent of the community at station 8 to 84.9 per cent at station 42. Dipterans made up a larger percentage of the population at downstream stations, however, the pattern was not clear cut.

The number of species taken in quantitative samples ranged from

Table 11. Benthic macroinvertebrates per m^2, Whetstone Creek. Means 2-3 samples taken between 1 June and 30 August 1966.

	Station					
	8	12	21	30	36	42
Coleoptera						
Corixidae						2
Dytiscidae					7	
Halipidae				2		
Heterlimnius sp				17	3	
Hydrophilidae	1					
Hydroporus sp					2	
Psephenus sp	1		1	1		
Diptera						
Ceratopogon sp			2	5		
Chironomids						
Calopsectra dives	2				2	
Calopsectra sp	2					3
Chironomus decorus			31	7	3	10
Microtendipes sp A	6	3				
Microtendipes sp B	1		2			
Pelopia sp						2
Pentaneura sp			34	18	15	3
Polypedilum sp	1					
Stictochironomus sp A	18	3	3		2	48
Stictochironomus sp B	6	3	5	12	7	2
Tanytarsus sp					8	
Unknown	3	2	3		10	2
Ephydridae				2		
Eristalis sp			3			
Dolichopus sp						2
Phychoda sp				2		
Rhagionidae					2	
Simulium sp			2			
Ephemeroptera						
Caenis sp	4		40	17	23	2
Ephemera simulans	9					

Table 11. Cont'd.

	Station					
	8	12	21	30	36	42
Ephemeroptera						
Stenonema sp	2					
unidentified	3		1	2		2
Hirudinea			10	8		
Malacostraca						
Asellus sp					2	
Hyalella azteca		3	37	78	2	2
Mollusca						
Ferrissia rivularis			211	7		
Fossaria parva	2					
Goniobasis livescens	69	8		263	3	
Pisidium sp	1					
Physa gyrina			45	11	3	5
Sphaerium sp	7	367	8	106	5	
Megaloptera						
Sialis sp	2	7	4	22	13	3
Odonata				12		
Oligochaeta		13	82	236	63	495
Plecoptera	16	2		7		
Trichoptera						
Cheumatopsyche sp	*	*	*	*	*	
Hydropsyche sp	59	10	30	115	22	
Philopotamus sp	1					
Pycnopsyche gentilis	1	23				
unidentified	4		5	5	3	
Turbellaria				5		

*Present but not counted.

Table 12. Benthic macroinvertebrates per m^2, Whetstone Creek, Means of four samples taken between 1 June and 30 November 1965.

	Station					
	8*	12**	21	30**	36	42**
Coleoptera						
Psephenus herricki	57	45	3	3		
unidentified	7			3	12	
Diptera						
Chironomids (unidentified)	87	130	188	163	37	
Tabanus sp	13	25				
Tipula sp	7		1			
Ephemeroptera						
Neocloeon sp				53		
Baetis sp				53		
Tricorythodes sp				20		
unidentified					1	5
Megaloptera						
Sialis sp				13		
Plecoptera						
Isoper a sp	50		3		3	
unidentified				20		
Trichoptera						
Hydropsyche sp	67	345	8	170	25	
Helicopsyche borealis		30				
Rhyacophilidae	23	13		17		
Limnophilidae	20					
unidentified		10	3			
Mollusca						
Leptodea fragilis						5

Table 12. Cont'd.

	Station					
	8*	12**	21	30**	36	42**
Mollusca						
Sphaerium sp	100	270	33	143	56	
Goniobasis livescens	183	195	1	183	8	35
Physa gyrina	3	25	35	33	16	20
Ferrissia rivularis			3	7	11	
Gyraulus sp				7		
Lampsilis sp		65		7		
Musculium sp						
Oligochaeta						
Tubifex sp			344	130	10	225
Malacostra						
Hyalella azteca			201		8	
Turbellaria				3	1	
Hirudinea			6			

* Means of only 2 samples collected between 15 October and 23 November 1965.

** Means of only 2 samples collected between 1 June and 15 September 1965.

twelve at station 12 to twenty-five at station 30. Dipterans, particularly chironomids accounted for approximately 30-40 percent of the species at most stations. However, Caenis sp, Hydropsyche sp, Sphaerium sp, Goniobasis livescens, and oligochaetes usually accounted for the largest numbers of individuals at each station. Stictochironomus sp, Caenis sp, Sialis sp, Hydropsyche sp, Cheumatopsyche sp, Sphaerium sp, Oligochaetes, and Hyalella azteca were found at five of the six stations.

Microtendipes sp, Polypedilum sp, Stenonema sp, Pycnopsyche gentilis, and Fossaria parva were found only at headwater stations 8 or 12 where there were fewer organic sediments and the water was cleanest and well oxygenated. Dolichopus sp, Tanytarsus sp, Rhagionidae, and Pelopia sp were found only at downstream stations 36 and 42 where pooling and organic sedimentation were more extensive. Eristalis sp, Simulium sp, Ceratopogon sp, Ferrissia rivularis, planaria, and leeches were found only at stations 21 and 30, sites nearest the Mt. Gilead sewage outfall.

Goniobasis livescens were absent in 1966 at station 21 (sewage outfall) and only $3/m^2$ were found in 1965, however, they occurred in large numbers both above and below the station. Sphaerium sp were distributed in a similar pattern. Physa gyrina, on the other hand, were found in larger numbers at station 21 than at any other station.

Olentangy

The composition of the benthic macroinvertebrate community is shown in Tables 13 nnd 14. Based upon averages of all stations, molluscs accounted for 30.1 per cent, Malacostraca 28.4 per cent, Diptera 14.5 per cent, and Hirudinea 11.7 per cent of the community. Dipterans, particularly chironomids made up the largest percentage (31.1 per cent) of the community at headwater station 2, but accounted for only 8 per cent of the total at station 12, the site nearest the Galion sewage outfall. Farther downstream, they ranged from 24-33 per cent of the community at each station. Molluscs and scuds accounted for over 70 percent of the community at station 12. However, the scud Hyallela azteca was collected at all stations except No. 66. Oligochaetes were found at all stations, accounting for as many as 50 percent of the total organisms at station 25. Mayflies accounted for less than 10 per cent of the community at every station except the lowermost, No. 66, where they made up 38.6 per cent of the organsims, the largest percentage of any group at that station. Caddisflies, mayflies, alderflies, and planaria were absent at station 12 (sewage outfall), but occurred at stations both above and below this major source of organic pollution.

The number of species taken in quantitative samples ranged from twelve at station 25 to thirty-nine at station 2. Heterlimnius sp, Stictochironomus sp, Pentaneura sp, oligochaetes, Hyalella azteca, and leeches were found at most stations. Berosus sp, Silpha sp, Hydrous sp, Ectopria sp, Hydroporus sp, Simulium sp, Tanytarsus sp, Ceratopogon sp, Hydrobaeninae, Glyptotendipes sp, Ferrissia rivularis, and Helisoma anceps usually were found only at headwater stations 2 and 12. Caenis sp, Ephemera simulans, and Potamanthus sp occurred only at downstream stations 54 and 66. Berosus sp, Silpha sp, Simulium sp,

Table 13. Benthic macroinvertebrates per m^2, Upper Olentangy River. Means 2-3 samples taken between 1 June and 30 August 1966.

	Station					
	2	12	25	36	54	66
Coleoptera						
Berosus sp		3				
Ectopria sp	1					
Elmidae	1					2
Dryopidae	2					
Haliplidae	2	8				
Heterlimnius sp	55		3	17	15	7
Hydrophilidae	2					
Hydroporus sp	1	2				
Hydrous sp	1					
Psephenus sp	189					1
Silpha sp		2				
unidentified	28	11	3	9	3	14
Diptera						
Ceratopogon sp	1	20				
Chironomids						
Calopsectra dives		15				2
Calopsectra sp	7			5		
Chironomus decorus	22	62	8		3	
Cryptochironomus sp	8			2		3
Glyptotendipes so		73				
Hyrobaeninae		300				
Microtendipes so A	120		8	96		13
Microtendipes sp B	3			3		2
Pentaneura sp	32	16		16	8	21
Polypedilum sp	27					6
Stictochironomus sp A	172		95	22	13	4
Stictochironomus sp B	33	3	3		8	3
Tanytarsus sp		2				
unknown 1				13	2	
unknown 2	15	17		3	4	7
Eriocera sp	11				2	2

Table 13. Cont'd.

	Station					
	2	12	25	36	54	66
Ephemeroptera						
Caenis sp					6	57
Ephemera simulans						8
Hexagenia atrocaudata			3	5	3	10
Potamanthus sp						6
Stenonema sp	17					6
unidentified				8		2
Hirudinea	5	946	3	9		1
Malacostraca		8				
Hyalella azteca	178	2250	70	110	3	
Mollusca						
Ferrissia rivularis	4	2				
Fossaria parva	2			2		
Goniobasis livescens	251			5		
Gyraulus sp	1					
Helosoma anceps	3	8				
Physa gyrina	20	2393	5			
Sphaerium sp	75			9		
Unio sp				2		
Megaloptera						
Sialis sp	7			5	5	9
unidentified						1
Odonata						
Libellula sp		2		8		
unidentified	2	5	3		3	3
Oligochaeta	23	110	215	252	30	4

Table 13. Cont'd.

	Station					
	2	12	25	36	54	66
Plecoptera						1
Trichoptera						
Cheumatopsyche sp	*			*	*	*
Helicophyche borealis	48					
Hydropsyche sp	57			46	8	19
Pycnopsyche gentilis				2		4
unidentified						3
Turbellaria						
Curtisia sp	4				2	

* Present but not counted.

Table 14. Benthic macroinvertebrates per m^2, Upper Olentangy River. Mean of two samples taken between 1 September and 15 October, 1965.

	Station				
	12	25	36	54	66
Coleoptera	5		300		
Diptera					
Chironomids (unidentified)					40
Tabanus sp	5				
Ephemeroptera					
Polymitarcys sp				5	25
Trichoptera					
Hydropsyche sp	5				
Helicopsyche borealis			280		
Rhyacophilidae			10		
Mollusca					
Sphaerium sp	25	20	1480	80	25
Goniobasis livescens		5	15	95	20
Physa gyrina	670	5			
Helisoma anceps	5				
Oligochaeta					
Tubifex sp	55	280			
Malacostraca					
Hyalella azteca	200				
Turbellaria					
Curtisia sp	105		370		

Tanytarsus sp, Hydrobaeninae, and Gluptotendipes sp, were collected only at station 12, the site nearest the Galion sewage plant outfall. Also unusually large numbers of Ceratopogon sp, Chironomus decorus, Hydrobaeninae, Glyptotendipes sp, Physa gyrina, Hyallella azteca and leeches were found at the latter station.

Heterolimnius sp, Stictochironomus sp A, Microtendipes sp, Sialis sp, Hydropsyche sp and Cheumatopsyche sp were absent from station 12 and sometimes No. 25, but were present in large numbers both above and below these stations.

Sandy Run-Lake Hope

Benthic macroinvertebrates per m^2 are shown in Table 15. The greatest abundance of organisms occurred in Lake Hope and station one below the lake. The widest diversity of species was found at unpolluted headwater station 5, in addition to Lake Hope and station 1. Chironomide were the most abundant organism at all stations except in Lake Hope which contained larger numbers of the culicid, Chaoborus sp. In addition to the chironomids, biting midges, the alderfly, Sialis sp, and aquatic earthworms were found at polluted and unpolluted stations. However, except for Sialis sp, they were reduced considerably in number in polluted reaches.

In Lake Hope, dragonflies, damselflies, and nematodes were common, in addition to Chaoborus and the chironomids. Mayflies and stoneflies were restricted almost completely to unpolluted station 5, while molluscs and caddisflies were conspicuous by their absence at nearly all stations.

Table 15. Benthic macroinvertebrates per m^2, Sandy Run - Lake Hope, annual means 1965-66.

	Station				
	1	LH	3	4	5
Coleoptera					
Donacia sp		2			
Dyticidae	3	2			1
Haliplus sp				1	
Hydroporus sp	3				
unidentified	1				
Diptera					
Ceratopogonidae	23	28	1	1	26
Chaoborus sp		656			
Chironomids	495	264	192	202	67
Erioptera sp					2
Limnophila sp					3
Rhaphidolabis sp		1			
Tabanus sp		1			
Tipulidae	1			1	16
unidentified	1	5		1	7
Ephemeroptera					
Heptagenia sp					3
Hexagenia sp		1			
Isonychia sp					19
Paraleptophlebia sp					1
Rhithrogena ap					4
Hemiptera	1		1		
Megaloptera					
Sialis sp	10	6	27	1	1
Odonata					
Coenagrion		1			
Epicordulia sp		1			
Gomphus sp	2	11			

Table 15. Cont'd.

	Station				
	1	LH	3	4	5
Odonata					
Hagenius sp	1				
Helocordulia sp	3	1			
Ischnura sp	2	34			
Lestes sp		14			
Macromia sp		1			
Nasiaeschna sp	1				5
Nehalennia sp		1			
Plecoptera					
Isogenus sp					19
Leuctra sp	1				
Nemoura sp					55
Trichoptera					
Dicosmoecus sp			4		
Hydropsyche sp	1				
Limnephilus sp	3		1		
Philopotamus sp	8				4
Mollusca					
Gyraulus sp	1				1
Pisidium sp					1
Oligochaeta	4	16		1	35
Nematoda	16	65			1
Total	585	1111	226	208	272

Honey Fork Area

Benthic macroinvertebrates per m^2 are shown in Table 16. The widest diversity and greatest abundance of organisms occurred at unpolluted station 2. Nematodes, chironomids, aquatic earthworms, and *Physa sp* were the most abundant organisms at this station. Biting midges and chironomids were the only organisms found at both polluted and unpolluted stations. Surprisingly, *Sialis sp* was collected at all polluted stations, but never at unpolluted station 2. Molluscans, dragonflies, and most caddisflies were restricted to unpolluted station 2.

Downstream areas of the main channel of Raccoon Creek were sampled only twice for benthic macroinvertebrates. The results are shown in Table 17. Very few species occurred at these stations, but occasionally large numbers of chironomids and aquatic earthworms were found.

Table 16. Benthic macroinvertebrates per m^2, Honey Fork area, annual means 1965-66.

	Station				
	W. Br. 1	Honey Fk. 2	Raccoon Cr. 3	W. Br. 4	E. Br. 5
Coleoptera					
Dytiscidae	2				
Hydrophilus sp		2			
unidentified		2			
Diptera					
Amnicia sp					2
Anthomyiidae		2			
Bibio sp		2			
Ceratopogonidae	7	15	177	12	17
Heleidae					
Chironomids	192	372	61	487	12
Silphidae		5			
Tabanidae		2			
Tipulidae		2		2	
Hemiptera			2		
Lepidoptera					
Pyralididae		3			
Malacostraca		2			
Mollusca					
Gyraulus sp		6			
Physa sp		35			
Planorbidae		2			
Nematoda		1153		41	
Megaloptera					
Sialis sp	37		30	82	2
Oligochaeta		52	5	5	
Odonata					
Hagenius sp		2			

Table 16. Cont'd.

	Station				
	W. Br./ 1	Honey Fk./ 2	Raccoon Cr./ 3	W. Br./ 4	E. Br. 5
Odonata					
Libellula sp		5			
Trichoptera					
Dicosmoecus sp		8		2	
Halesus sp		2			
Philopotamus sp		2			
Ptilostomis sp					2
Total	238	1676	275	631	35

Table 17. Benthic macroinvertebrates per m², stations 8,9, and 10, Raccoon Creek (main channel), 1965-66.

	Station 8		Station 9		Station 10	
	4/3/66	6/4/66	4/3/66	6/4/66	4/2/66	6/3/66
Ceratopogonidae					20	
Chironomids	110	1085	10	70	70	
Donacia sp		10				
Lestes sp					10	
Notonecta sp				30		
Oligochaeta			160	70	65	
Sialis sp	10		70	30	20	

DISCUSSION AND CONCLUSIONS

Algae

Numerous publications have appeared in the past 60 years relating various species of algae to different levels of pollution in streams (Kolkwitz and Marrson, 1908; Hentschel, 1925; Naumann, 1925, 1932; Butcher, 1947; Liebmann, 1951; Schroeder, 1939; Hayren, 1944; Patrick, 1949). Patrick (1965), in summarizing the results of research in this field, states, "It is apparent that certain species of blue-green algae, red algae, green algae, and diatoms may be useful in qualitatively indicating certain chemical and physical conditions of the water as they relate to pollution and that most of these species are tolerant to various types of pollution rather than indigenous to them. The most reliable method in considering algae as indicators of pollution is to study the algal community as a whole and consider the kinds of species, relative sizes of the populations of the various species, and numbers of species. Furthermore, if one wants to compare the algal flora in various areas it is necessary to base conclusions on similar segments of the algae community."

Whetstone-Olentangy

Based upon sewage discharge rates, chemical analyses (phosphates, nitrates and oxygen), and observations of organic sedimentation, the Olentangy River is more heavily polluted with organic materials than is Whetstone Creek. Analysis of the algal community appears to support this conclusion (Tables 7 and 8).

An equal number of algal genera occurred in the two streams, but differences were noted in the types of genera represented. Although diatoms and green algae were dominant in both streams, greens, blue-greens, euglenoids, and dinoflagellates were more abandant in the Olentangy than in Whetstone Creek. The number of diatom genera was approximately equal in the two streams, but our results confirm reports that species of Nitzschia, Diatoma, and Surirella (Round, 1962) are frequently associated with organically enriched water. These organisms were more abundant in the Olentangy River than in Whetstone Creek. The greater abundance of blue-green algae in the Olentangy, particularly species of Oscillatoria, Anabaena, and Merismopedia, also are indications of greater organic enrichment. The presence of flagellated forms such as euglenoids, dinoflagellates, and certain green algae may reflect the lower stream gradient of the Olentangy River. Pools resulting from the low gradient provide a more suitable physical habitat for growth of flagellated organisms and green algae. In addition to a slower current, there is less shading of the surface, permitting greater illumination of the water column. Green algae and especially dinoflagellates require relatively high light intensities for optimal photosynthesis (Ryther, 1956). Hynes (1967) believes that shade is the most important factor limiting primary production in most streams.

In water polluted by brines, a whole range of halophilic diatoms have been reported including Caloneis amphisbaena, Amphora, Aphiprora, Bacillaria paradoxa, Nitzschia obtusa, and Cylindrotheca gracilis (Round, 1965). Our results neither support nor reject these reports. We found species of Cylindrotheca, Caloneis, and Nitzschia in both streams, but not limited to brine polluted areas, while species of Amphora, Aphiprora, or Bacillaria were not observed in either stream. Only seven genera of algae were collected in Whetstone Creek that did not occur in the Olentangy River, but none of these appeared restricted to brine polluted areas. Reimer (1965) compiled chemical and diatom distribution data from numerous sources for five well defined species: Melosira varians C. A. Ag., Nitzschia amphibia Grun., Navicula confervacea Kutz., Cymbella tumida (Bréb) V. H., and Navicula ingenua Hust. His results indicate that all organisms except C. tumia occur rather frequently at chloride concentrations considerably higher than we have encountered. Several of the diatoms occurred at chloride concentrations as high as 10,000 ppm. We did not observe chlorides greater than approximately 500 ppm.

Considering the diversity and abundance of algae in Whetstone Creek, the chemical data, and comparisons of each of these to the Olentangy, it appears unlikely that chlorides have caused severe changes in the algal flora to date. Subtle changes in community structure may be occurring, but if continued under present conditions several years may be required before the alterations in structure are perceptible.

Raccoon Creek

Peridinium inconspicuum Lemm, and Closterium acutum (Lyngb) breb., were dominant phytoplankters in Lake Hope and have not been previously reported from acid mine waters. These organisms were homogeneously distributed to a depth of six meters in Lake Hope. P. inconspicuum bloomed in early summer when Secchi disc readings were highest, indicating a deep light penetration. Turbidities were always low in Lake Hope (Table 5). Secchi readings ranged up to 4.3 meters, much greater than most non-acid Ohio lakes. These high light intensities are probably favorable for the growth of dinoflagellates such as *P. inconspicuum* which have a high light requirement for optimal photosynthesis (Ryther, 1956). *P. inconspicuum* is not a common phytoplankter in eutrophic lakes with high turbidities and low light penetration. Thus in Lake Hope where acidic conditions limit the growth of most algae, illumination is high, creating a favorable environment for the acid tolerant *P. inconspicuum.* As the *P. inconspicuum* population increases, self shading occurs, limiting its growth and probably altering the nutrient spectrum in the lake. These conditions then become favorable for the acid-tolerant *C. acutum* which blooms in late summer. Over winter, all organisms are reduced in number, clearing the water column and again creating an environment favoring the growth of *P. inconspicuum.*

Cyanophyta are typically absent from acid-mine waters. We encountered small *Oscillatoria sp* and *Anabaena sp* populations in the relatively unpolluted Honey Fork tributary flowing at average pH values of 5.0, but never in acidities lower than pH 4.0. The latter observations were very rare and probably represented a temporary situation.

Unpolluted Honey Fork station 2 and Sandy Run stations 1 and 5 contained a greater diversity of algal genera than polluted stations (Figs. 8, 9). Lake Hope had a low diversity of genera, but greater abundance of organisms per ml than stream stations. These results are not unexpected, confirming the observations of other investigators of acid-mine waters (Lackey, 1939; Joseph, 1953; Warner, 1965).

In general, the presence of Lake Hope on Sandy Run appears to have a beneficial effect on the flora and fauna of the stream (Figs. 9, 10). Although Lake Hope is unproductive compared to the other unpolluted Ohio Lakes, physiological processes operating within the lake improve the physico-biological conditions sufficiently to permit growth of a more abundant and diverse flora at station 1 below the lake spillway. In fact, the diversity and abundance of organisms and chemical conditions are essentially similar to unpolluted station 5 (Figs. 9, 10, 11, 12). It is doubtful that Sandy Run would recover as quickly without the reservoir. Similar improvement was not noted in downstream reaches of other polluted tributaries which do not contain reservoirs (Fig. 8).

The reasons for the improvement in water quality are not clearly understood but probably occur via several mechanisms operating concurrently. For example, photosynthesis increase pH by removing CO_2 (carbonic acid) from the water. Carbon dioxide also may be removed from the stream by the conversion of carbonate hardness to non-carbonate sulfate hardness. Toxic quantities of ferrous iron (and perhaps other metallic cations) may be removed upon oxidation by bacteria and precipitation as ferric hydroxide (Hynes, 1960). In a lake, the precipitate may be buried in other sediments and thus removed from the water. This process would be difficult to duplicate in flowing water.

Algal nutrition and metabolism have not been studied extensively under field conditions especially in acid waters. The interactions of living systems with the unusual ionic ratios found in acid-mine water would be very complex. Hydrogen ions and metallic cations undoubtedly alter the reactive sites of enzymes, in-

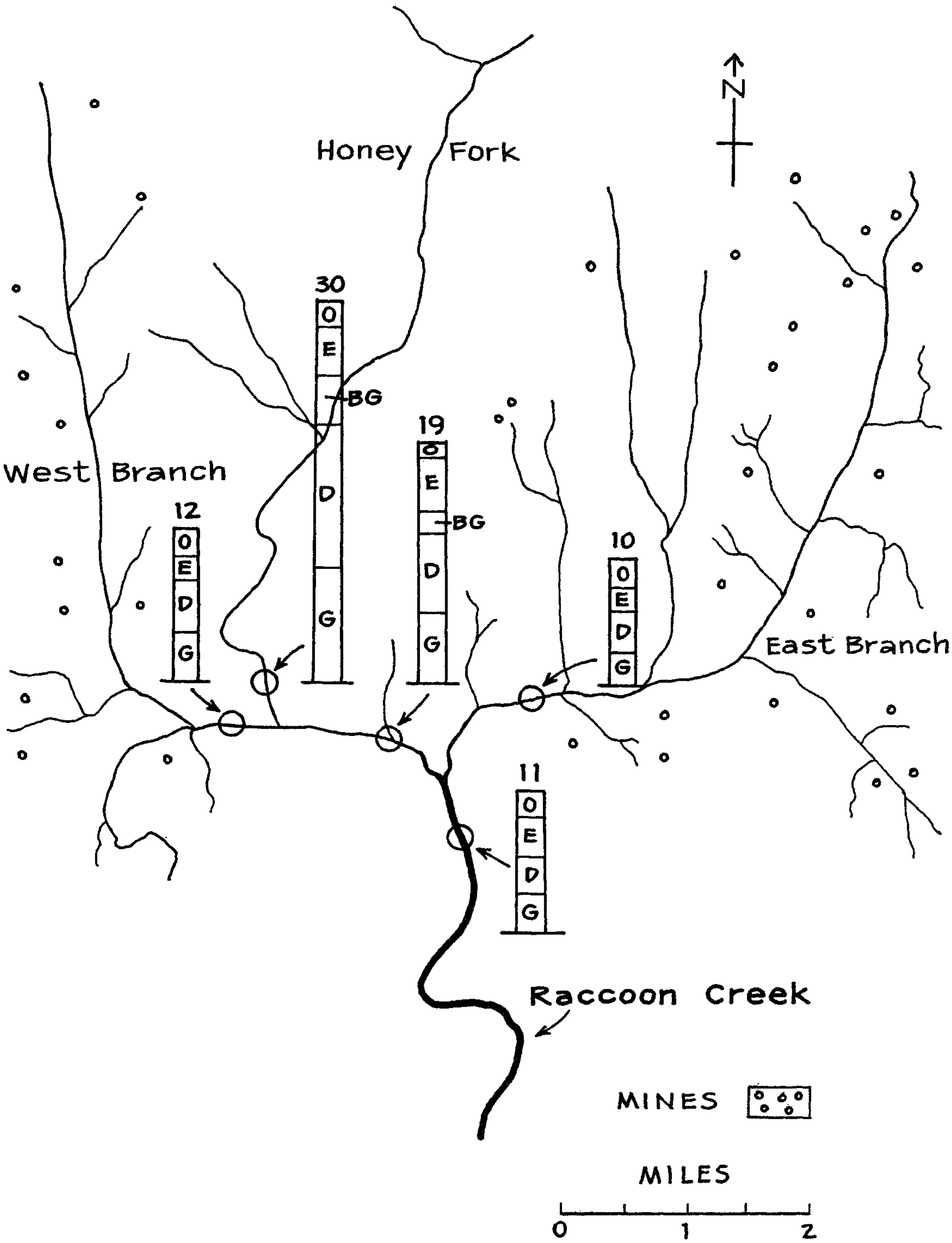

Fig. 8. Comparison of the number of algal genera occurring at 5 stations in the Honey Fork area of Raccoon Creek. G=green algae, D=diatoms, E=euglenoids, BG=blue greens, and O=other. Number at top of each column indicates the number of genera collected during an annual cycle.

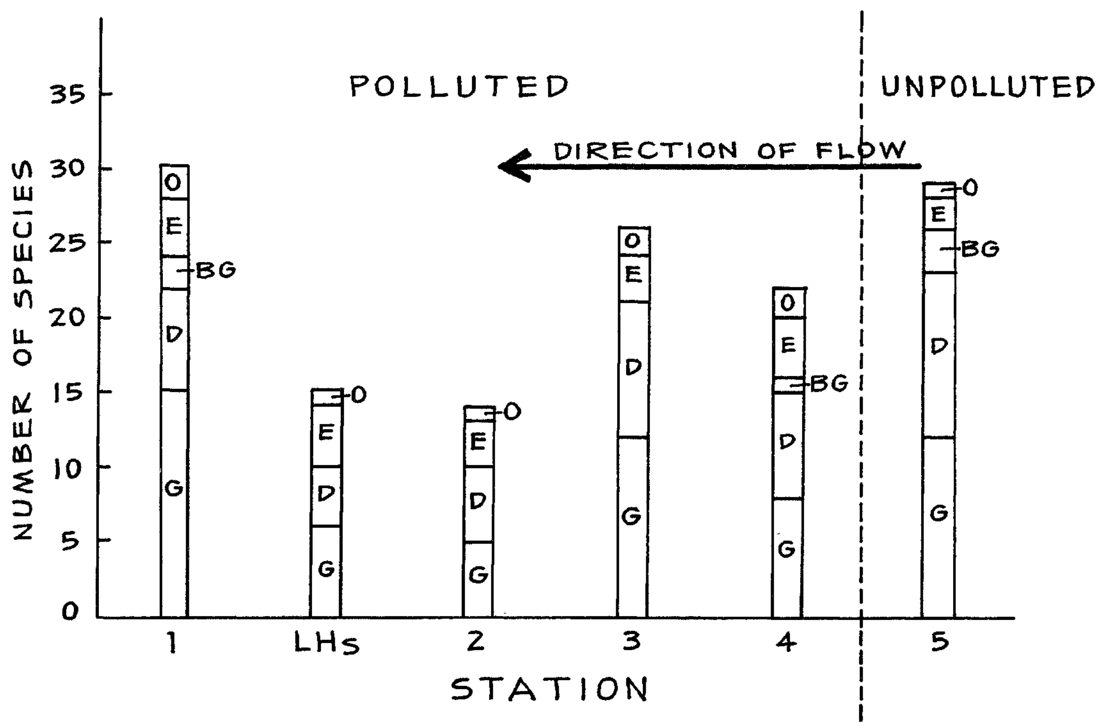

Fig. 9. Comparison of the number of algal genera occurring at 6 stations on Sandy Run-Lake Hope tributary of Raccoon Creek. G=green algae, D=diatoms, BG=blue-greens, E=euglenoids, and O=other. Annual means.

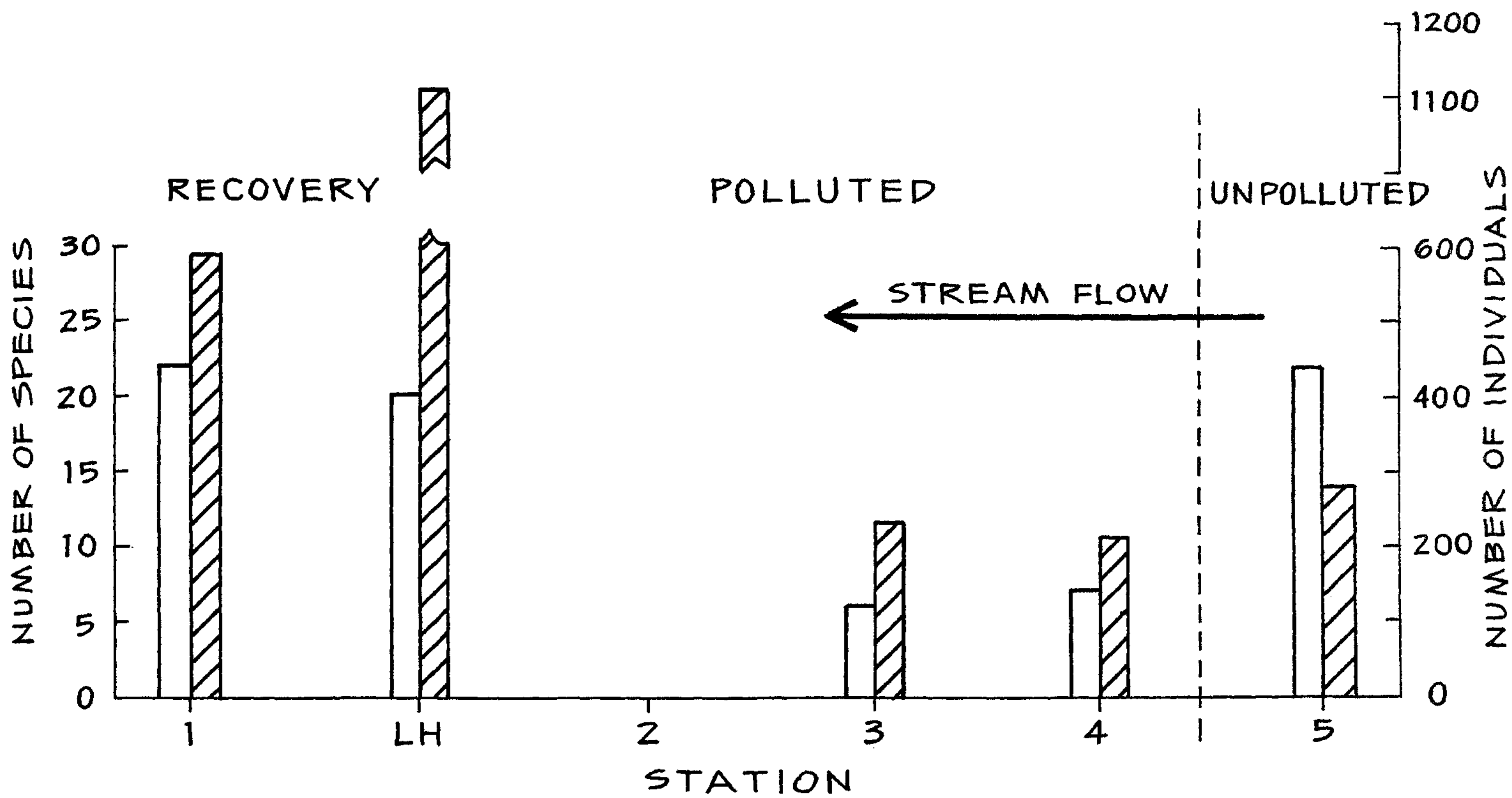

Fig. 10. Comparison of the number of species and the number of benthic macroinvertebrates per m^2 at 6 stations on Sandy Run-Lake Hope tributary of Raccoon Creek. Annual means.

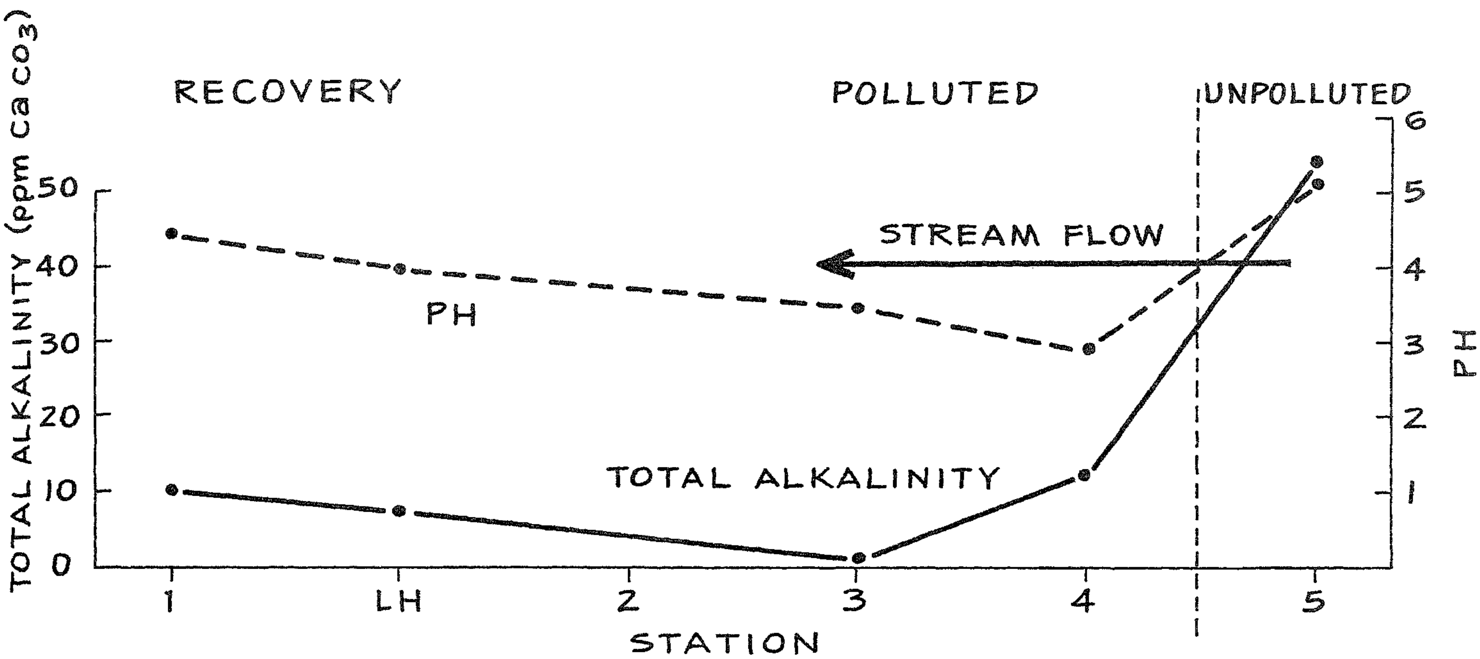

Fig. 11. Comparison of pH and total alkalinities at 6 stations on the Sandy River-Lake Hope tributary of Raccoon Creek. Annual means.

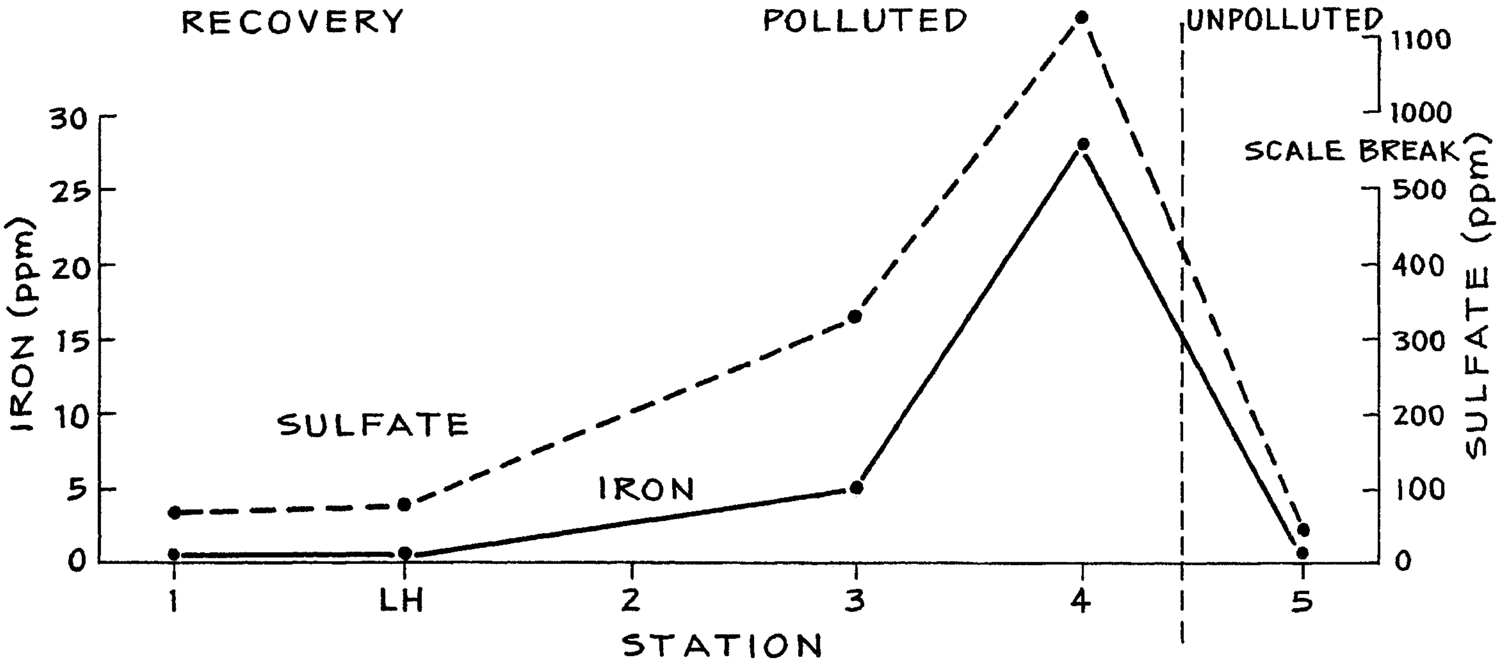

Fig. 12. Comparison of sulfates and iron concentration at 6 stations on the Sandy River-Lake Hope tributary of Raccoon Creek. Annual means.

fluencing not only reaction rates but the nature of end products. These processes, in turn, can be expected to affect the distribution of organisms at all higher trophic levels.

Cell membrane permeability is influenced markedly by salt concentration and pH. Ferrous salts (and others) usually decrease the permeability of algal cell walls to penetration of solutes into cells. Respiration is increased and photosynthesis decreased. Low pH accelerates the exosmosis of sugars, (Stadelmann, 1962) and auxins (Conrad and Saltman, 1962) from certain algae. Intracellular phosphate levels increase in alkaline solutions (Kuhl, 1962), but the solubility of iron salts, (and others) is increased by an increase in acidity. Small (1946) found that *Chlorella* grew well at pH 4.6-7.0, but when iron was kept in solution the optimum pH was 7.5.

Fogg (1965) noted that adding nitrogen as an ammonium salt lowered pH, but the addition of nitrate nitrogen increased pH. Nitrogen deficiencies reduce photosynthetic rates and inhibit cell division, however, further photosynthesis causes cell enlargement. We observed large cells of *P. inconspicuum* following a reproductive maxima in Lake Hope, indicating a possible nitrogen deficiency. The concentration of nitrogen also may influence sexuality in algae. Nitrogen depletion hastens gamete formation increasing the zygote yield of certain algae (Coleman, 1962). Burley (1955) increased the growth of *Chlorella* in acid-mine water by adding nitrates and phosphates, but water which had been neutralized without additional N or P was poor media for algal growth. All waters examined in Raccoon Creek and its tributaries were poor in nitrates and phosphates. Inhibition of saprophytic bacteria and fungi by the acid water slows the rate of decomposition of organic matter, thus retarding the generation of oxidized N and P. The slow decomposition of leaves, logs, and dead fish are clearly evident in Lake Hope and Raccoon Creek.

Macroinvertebrates

Many investigators have published list of "indicator" organisms associated with various qualities of water (Richardson, 1921; Mackenthun et. al. , 1956; Weston and Turner, 1917; Gaufin, 1958; Surber, 1953; Wilson, 1953), but there is lack of agreement as to the true status of many forms. According to Gaufin (1958), this lack of agreement is a result of regional differences in species, the environment, the mechanism of evaluation, and types of pollution, etc. Many organisms occurring in large numbers in polluted areas also may be found in smaller number in cleaner sections. For thse reasons , the abundance as well as the species composition of a benthic community are important in assessing pollutional levels.

Approximately sixty-two species of macroinvertebrates were collected in each of the two streams. Thirteen macroinvertebrates were found in Whetstone Creek but not in the Olentangy River including Corixidae, *Eristalis*, *Dolichopus sp*, Ephydridae, Rhagionidae, *Pelopia sp* , *Pisidium sp*, *Lampsilis sp*, *Musculium sp*, *Baetis sp*, *Tricorythodes sp*,*Neocloen sp*, and *Corydalis sp*. None of thse organisms occurred in large numbers, however. Fifteen species were collected in the Olentangy River but not in Whetstone Creek including Berosus sp, Silpha sp, Hydrous sp, Ectopria sp, Elmidae, Dryopidae, Heliodae, Eriocera sp, Hydrobaeninae, Glyptotendipes sp, Cryptochironomus sp, Potamanthus sp Helisoma anceps, Gyraulus sp, and Polymitarcys sp. Of these , only Hydrobaeninae and Glyptotendipes sp , were abundant in the Olentangy River, occurring primarily in the most heavily polluted zone. Chironomids, particularly the hemoglobin containing species have been regarded by many investigators as pollution tolerant. Walshe (1947, 1950) has shown that the hemoglobin possessed by midge larvae such as Chironomus riparius and Chironomus plumosus serves in both storage and transportation of oxygen. This adaptation permits these organisms to continue feeding in low oxygen tensions enchancing their chances of survival in septic regions.

Many species of chironomids are intolerant of organic pollution (Gaufin, 1958) . Stictochironomus sp, and Microtendipes sp in particular, were eliminated or occurred in reduced numbers at polluted stations in both streams, but were present upstream and downstream from these locations.

In addition to the tolerant Chironomids, large numbers (over 100 m^2) of Physa gyrina, Hyalella azteca, Tubifex sp, and leeches also were assocated with the most heavily polluted station on the Olentangy River. In Whetstone Creek, only Ferrissisa rivularis was found in numbers larger than $100/m^2$ at the organically enriched station 12. Tolerant chironomids, Physa, H. azteca, Caenis sp , and aquatic earthworms also were abundant in polluted areas of Whetstone Creek. Certain chironomids, snails, tubificids, and leeches are regarded by most workers as indicative of organic pollution. In Lytle Creek

Ohio, Gaufin and Tarzwell (1956) found the pulmonate snail, Physa integra, to be a large component of the macroinvertebrate population in polluted reaches. Its ability to obtain atmospheric oxygen directly enabled it to take advantage of the abundant food supply in polluted areas.

Associations of myflies, stoneflies, and caddisflies usually indicate clean water, and their absence may denote organic pollution as long as the habitat is suitable (Gaufin and Tarzwell , 1956). The most immediate effect of organic pollution on the environment is the lowering of dissolved oxygen. Most forms of aquatic life cannot tolerate dissolved oxygen below 3-5 ppm for long periods of time, although for short periods as little as 2 ppm may be tolerated (Patrick, 1962). Dissolved oxygen appears adequate for most organisms in both Whetstone Creek and the Olentangy River at the stations sampled. However, the so-called polluted stations (0-12 and W-21), located 1-2 km below the sewage outfalls, probably contained higher dissolved oxygen levels than upstream reaches nearer the outfalls.

Mayflies and caddisflies were present at Whetstone station 21 despite organic pollution, but in the Olentangy River only a few caddisflies ($5/m^2$) were present at the pollution sites. Stoneflies were not common in either stream, even at unpolluted stations, but mayflies and caddisflies were abundant in both streams in unpolluted reaches.

Mathis (1965) found distinct differences in species composition above and below sources of oil field brines in Black Bear Creek, Oklahoma. He noted 47 species upstream from the brine outfall and 31 downstream, but only one species, Ferrissia shimeki occurred both above and below the pollutions source. Another species, Hyalella azteca, an animal usually associated with clean water, was found at the unpolluted station. In contrast, we found *H. azteca* and *Ferrissia rivularis* most abundant at organically enriched stations.

F. rivularis was also abundant in Whetstone Creek at station 21, a site receiving large amounts of both organic and brine wastes. Mathis compared Black Bear Creek with one of its unpolluted tributaries and found large numbers of *Sphaerium transversum*, mosquito larvae, phantom midges, alderflies, and leeches in the tributary, but these organisms were rare or absent in the brine polluted stream. Clemens and Finnell (1955) found only four species in a brine polluted stream in southern Oklahoma where chloride concentration was 13,000 to 25,000 ppm, but thirteen species at chloride levels below 1,000 ppm. We did not encounter chloride concentrations greater than approximately 500 ppm, values unlikely to cause abrupt changes in benthic communities.

Index of Community Diversity

Several attempts to interpret community structure quantitatively from the relationship between numbers of individuals and species present have resulted in various indexes of diversity (Fisher, Corbett, and Williams 1943; Preston 1948; Patten 1962; Wilson and Dorris 1966 ; Margalef 1951). The species diversity index, $\bar{H} = -\sum_{i=1}^{m} (n_i/N) \log_2 (n_i/N)$, where N = total number of individuals, n_i = number of individuals of species i, and m = number of species /unit area appears especially useful for analyzing benthic macroinvertebrate communities (Margalef 1956; Patten 1962). The advantages and disadvantages of $\bar{H}$ in assessing pollutional levels have been discussed by Wilm and Dorris (1966). These investigators believe that $\bar{H}$ is a particularly good measure of community structure because it is demensionless and numbers or biomass in any units can be used.

Clean streams are usually characterized by many species of equal abundance and hence have high diversity indexes. However, streams polluted with organic wastes have low diversity indexes because many intolerant species are eliminated and a few tolerant forms flourish in the absence of competition. From the small number of studies available (Wilm and Dorris 1966; Mathis 1965; Harrel 1966), $\bar{H}$ values greater than 3 are usually obtained in clean water areas, values 1 - 3 in areas of moderate pollution, and values less than 1 in heavily polluted areas.

In Whetstone Creek and the upper Olentangy River, most $\bar{H}$ values averaged between 1 and 3 suggesting "moderate" pollution. Average $\bar{H}$ values greater than 3 occurred only at headwater station 2 on the Olentangy River. Values less than 1 were not encountered on either stream (Fig. 13). $\bar{H}$ was usually lowest immediately

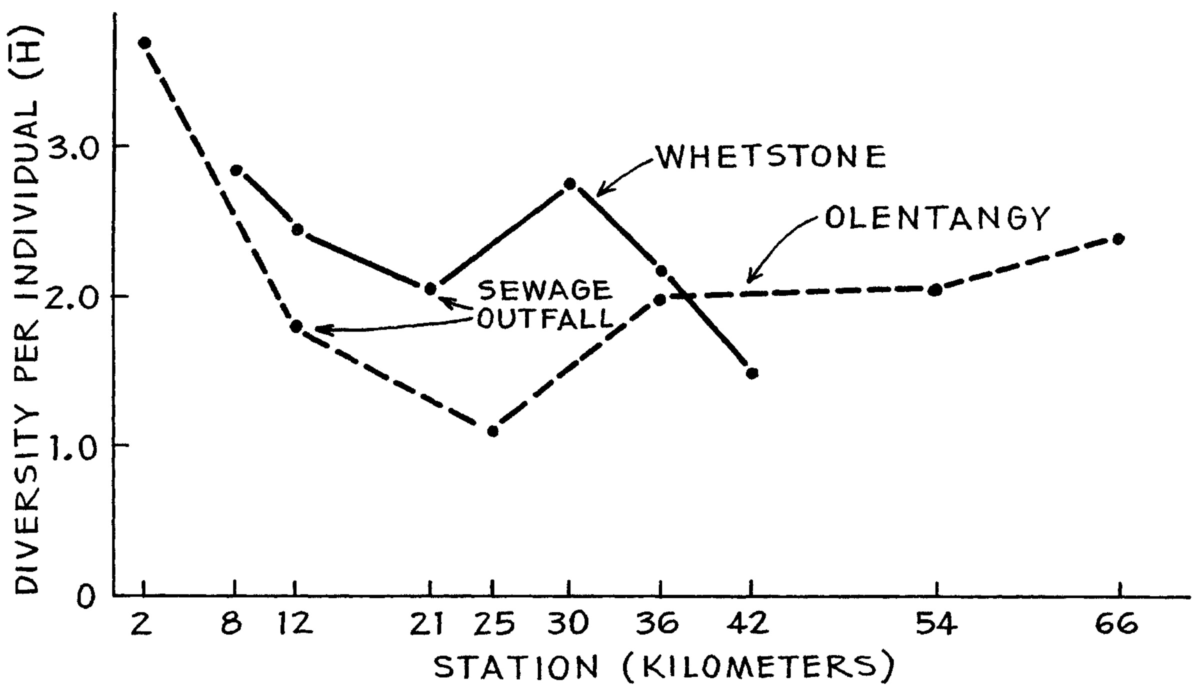

Fig. 13 Comparison of diversity/individual indexes in Whetstone Creek and the upper Olentangy River. Summer 1966.

below sewage outfalls on the Olentangy River and Whetstone Creek, however, on Whetstone Creek low $\bar{H}$ also occurred at the lowermost station 42. In contract to the latter, Wilm and Dorris (1966), Mathis (1965), and Harrel (1966) usually found $\bar{H}$ increased in downstream areas of certain Oklahoma streams. This was interpreted as "improved" stream conditions by these investigators. On Whetstone Creek, the low species diversity at station 42 probably reflects the low diversity of habitat in this area. Station 42 is near the Delaware Reservoir and has no riffles but rather contains extensive pools with heavily silted bottoms. The result is a uniform habitat that probably limits development of a diverse benthic community. This latter suggestion presents a possible exception to the generalization that clean areas have high diversity indexes and aseptic regions have low diversity indexes.

There must be a similarity of natural habitat diversity in clean and polluted areas before species diversity indexes accurately reflect differences caused by organic pollution. Species diversity indexes probably are directly proportional to habitat diversity. Therefore, $\bar{H}$ accurately reflects the levels of organic pollution only to the extent that organic pollutants lower habitat diversity can be lowered by factors other than organic effluents such as a change in water level, velocity, and siltation among others.

Phosphates and dissolved oxygen, both of which reflect the presence of domestic wastes, correlates well with $\bar{H}$. Dissolved oxygen was lowest and phosphates usually highest at stations with lowest $\bar{H}$ values and nearest sewage outfalls (fig. 5, 6, 13). In downstream areas DO gradually increased and phosphates decreased correlating well with increased $\bar{H}$. One exception occurred on

Whetstone Creek where DO was relatively high at station 42 despite high phosphates and low species diversity. As noted above, the low diversity of habitat in this region may have limited the diversity of organisms. Good correlations between $\bar{H}$ and other chemical components of the stream water are not evident.

Raccoon Creek and Tributaries

Acid-mine water is characterized by a low diversity of species, similar to that observed in organic pollution (Fig. 14). However, tolerant organisms do not increase as abundantly in acid as in organically polluted water. In organically enriched streams, oxygen deficiency usually limits the number of species present. But if an organism has some adaptation whereby oxygen can be obtained from the atmosphere, then the organic environment is ideal for the population growth of the adapted organism. In acid waters, however, ionic imbalances interfere with metabolic activity, thereby limiting population growth regardless of the amount of oxygen or food available and the absence of competitors.

All waters investigated in Raccoon Creek and tributaries appeared to be extremely well oxygenated. The only exception may have been unpolluted Honey Fork station 2, although no measurement of dissolved oxygen taken at this station was less than 5.8 ppm. Despite this, the oxygenophilic, Sialis sp, was conspicuously absent at this station, but occurred abundantly at all other stations.

The diversity and abundance of macroinvertebrates in Lake Hope and station 1 supports our conclusions above (see discussion on algae) that the presence of a reservoir improves the physico-biological conditions of an acid stream more rapidly than it would recover if not impounded (Figs. 10, 14).

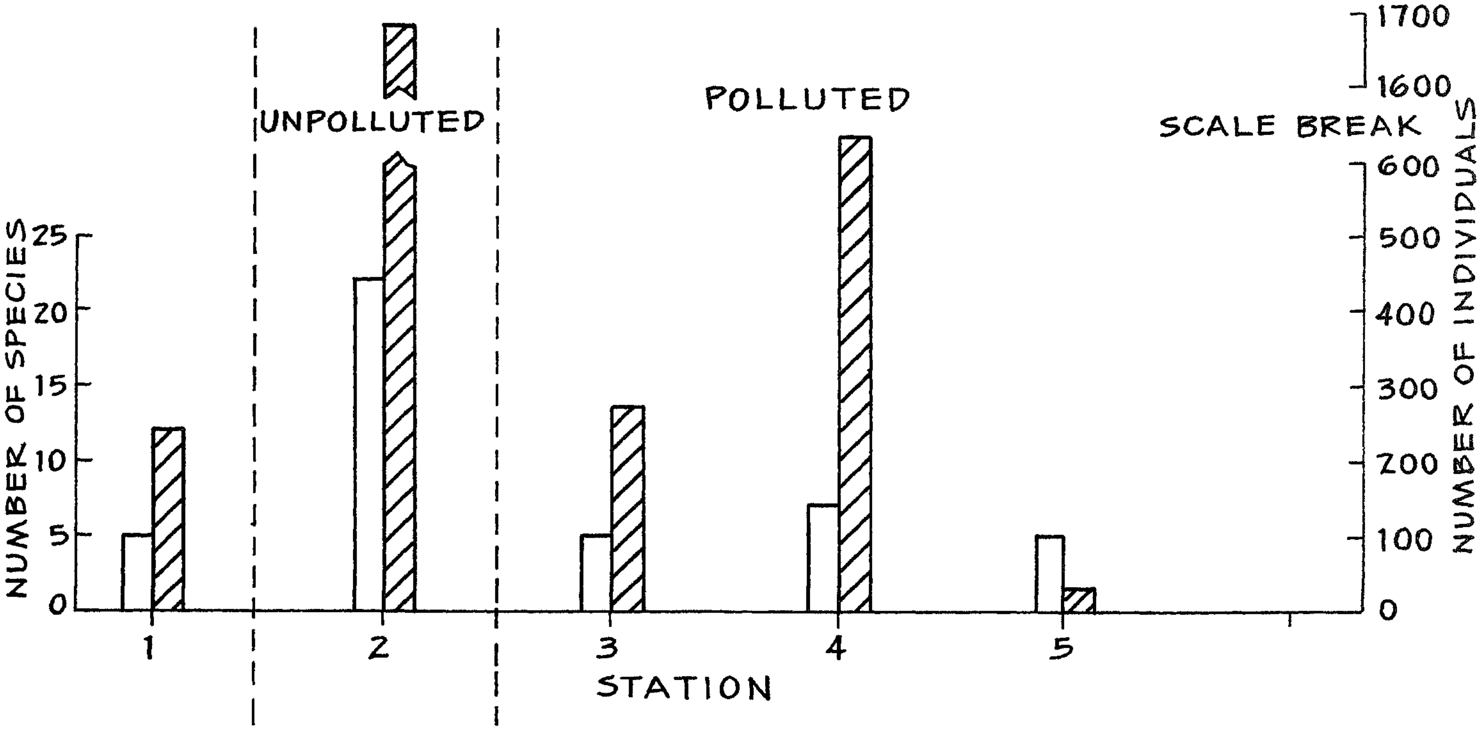

Fig. 14 Comparison of the number of species and number of benthic macroinvertebrate per m^2 at 5 stations in the Honey Fork area of Raccoon Creek. Annual means.

The midge larvae which tolerate oxygen-poor environments so well also are tolerant of acid-mine water. We are unaware of the physiological reasons for this, but the hemoglobin that they possess, enabling them to obtain and transport oxygen under low tensions, also may provide a buffering system capable of minimizing the deleterious effects of low pH or other ionic imbalances. We found *Chironomus decorus* and *Chironomus tentans* to be the dominant midges in both the acid polluted and unpolluted stations. These organisms contained large quantities of hemoglobin.

Harp and Campbell (1967) usually found *tendipies* (Chironomus) *plumosus* (linne) to be the sole chironomid established in strip-mine lakes of Missouri and Kansas with pH values lower than 6.0. These investigators tested the hypothesis that survival of *T. plumosus* in acid waters could be explained by their residence in substrates which were less acid than the overlying water. Analysis of the sediments, however, indicated that the differences in pH between the sediments and overlying water were insufficient to support their suggestion. No physiological mechanism was proposed.

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